STUDIES IN ACUTE LIVER FAILURE

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Doctor of Medicine

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2011

Declaration

I, Darren George Norman Craig, hereby declare that the work described herein has been composed by myself, and has not been submitted for any other degree or professional qualification.

28.11.11

Acknowledgements

I would like to thank my supervisors Dr Kenneth Simpson and Professor Peter Hayes for their tremendous support, advice, and good humour throughout this project.

I thank Mrs Anne Pryde and Mrs Patricia Lee for their technical support and refreshments; Janice Davidson and Kirsty Martin for database assistance; and Dr Gail Masterton for her help with patient recruitment. I also acknowledge the assistance of Dr Andrew Conway Morris with flow cytometry; Drs Simon Walker & Geoffrey Beckett for biochemistry input; and Dr Christopher Bellamy with histology.

Above all, I wish to thank my wife Philippa for her unwavering support whilst I undertook and completed this project, despite the significant geographical and personal hurdles involved.

For Pippa & James

Abstract

Acute liver failure (ALF) is a devastating condition with a high associated mortality rate. Paracetamol hepatotoxicity remains the leading cause of ALF in the developed world. The studies outlined in this thesis explore the current management of ALF, and systematically review the prognostic tests currently used in paracetamol-induced ALF. Using a database of over 900 acute liver injury patients, the impact of unintentional paracetamol overdose is retrospectively analysed, demonstrating a strong association between this mode of paracetamol overdose and adverse clinical outcomes, including the requirement for emergency orthotopic liver transplantation.

Current prognostic tests for severe paracetamol-induced hepatotoxicity have been criticised for their relatively low sensitivity, with the result that not all patients who might benefit from tertiary level care are identified. This thesis demonstrates that the development of the Systemic Inflammatory Response Syndrome (SIRS) or extrahepatic organ failure is strongly associated with death following paracetamol overdose. Due to their very high sensitivity in this condition, both the SIRS and Sequential Organ Failure Assessment scores have potential as future gatekeepers to improve the triage of paracetamol overdose patients, thereby delivering tertiary level care to those most likely to require emergency transplantation.

A greater understanding of the pathophysiological links between the initial hepatic injury and development of the SIRS could help to identify novel biomarkers for ALF, and help guide future therapeutic avenues. Using serum samples from a prospectively collected cohort of acute liver injury patients, this thesis identifies two novel biomarkers, serum ferritin and the long pentraxin PTX3, which show a strong association with outcome following paracetamol hepatotoxicity. These biomarkers illustrate the importance that the innate immune system plays in the pathogenesis of paracetamol-induced ALF, and identifies several exciting areas for future cellular and animal-based studies.

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Abbreviations

AFLP	Acute fatty liver of pregnancy
ALF	Acute liver failure
ALT	Alanine aminotransferase
APACHE	Acute Physiology and Chronic Health Evaluation
AUC	Area under the curve
AUROC	Area under the receiver operator characteristic
CBF	Cerebral blood flow
CI	Confidence interval
CIRCI	Critical-illness related corticosteroid insufficiency
CLD	Chronic liver disease
CMV	Cytomegalovirus
CPP	Cerebral perfusion pressure
CRP	C-reactive protein
DOR	Diagnostic odds ratio
EBV	Epstein-Barr virus
ELISA	Enzyme-linked immunosorbent assay
FFP	Fresh frozen plasma
HAART	Highly Active Anti-Retroviral Treatment
HAV	Hepatitis A virus
HBV	Hepatitis B virus

HC	Healthy control
HDV	Hepatitis delta virus
HE	Hepatic encephalopathy
HELLP	Haemolysis, elevated liver enzymes, low platelets
HEV	Hepatitis E virus
HSV	Herpes simplex virus
ICP	Intracerebral pressure
IL	Interleukin
INR	International normalised ratio
IQR	Interquartile range
KCC	King's College Hospital poor prognostic criteria
KCH	King's College Hospital
MARS	Molecular Absorbent and Recirculating System
MELD	Model for End-Stage Liver Disease
NAC	*N*-acetyl cysteine
OLT	Orthotopic liver transplantation
OR	Odds ratio
OTC	Over the counter
POD	Paracetamol overdose
PRR	Pattern recognition receptor
PT	Prothrombin time
PTX3	Pentraxin 3

RCT	Randomised controlled trial
ROC	Receiver operator characteristic
RRT	Renal replacement therapy
SAP	Serum amyloid P
SIRS	Systemic Inflammatory Response Syndrome
SLTU	Scottish Liver Transplantation Unit
SOFA	Sequential Organ Failure Assessment
VZV	Varicella zoster virus
WCC	White cell count

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Chapter 1: The current management of acute liver failure

1.1 Introduction

Acute liver failure (ALF) is a rare disorder characterised by catastrophic loss of liver cell function. It remains one of the most challenging medical emergencies, due to the multiorgan nature of the disease, the rapid evolution of the clinical condition, the need for multidisciplinary supportive interventions, and the requirement for the clinician to accurately prognosticate in order to best utilise orthotopic liver transplantation (OLT) as a life-saving treatment. Despite advances in supportive care, spontaneous survival without OLT is as low as 20%, therefore early recognition and prompt transfer of potential transplant candidates to tertiary centres with intensive care and liver transplantation expertise is vital.

1.2 Definition

ALF refers to the abrupt loss of hepatic cellular function in a patient without pre-existing liver disease, with the subsequent development of coagulopathy, jaundice and encephalopathy. In 1970, Trey and Davidson defined fulminant hepatic failure as a "potentially reversible condition, the consequence of severe liver injury, with an onset of encephalopathy within 8 weeks of the appearance of the first symptoms and in the absence of pre-existing liver disease".(Trey and Davidson 1970) However, since the initial symptoms of liver failure are frequently non-specific and open to subjective bias, ALF was redefined based upon the time taken to develop hepatic encephalopathy (HE) after the first appearance of jaundice; the terms 'hyperacute', 'acute', and 'subacute' liver failure referring to a jaundice-to-encephalopathy interval of 0- 7, 8-28, and 29-84 days respectively.(O'Grady, Schalm, Williams 1993) This distinction is useful in guiding prognosis since, paradoxically, the time to onset of encephalopathy is negatively correlated with outcome despite the increased incidence of cerebral oedema in hyperacute liver failure. Hepatitis A and B, paracetamol, and ischaemic insults typically present as hyperacute liver failure, and have a relatively good spontaneous survival rate of 36%, whereas idiosyncratic drug reactions and indeterminate causes present later, with only a 14% survival rate without OLT.(Gimson 1996) Severe acute liver injury, with elevated transaminases and coagulopathy, typically

precedes hyperacute liver failure but it is HE, the cardinal feature of the progression to ALF, which defines the condition and is of major prognostic significance.

1.3 Incidence and aetiology

The accurate reporting of ALF is hampered by the heterogeneous nature of the syndrome and by the lack of an International Classification of Diseases code for ALF. This means that the incidence is probably under reported but has been estimated at 2800 cases per annum in the US or approximately 3.5 deaths per million population.(Hoofnagle and others 1995; Polson and Lee 2005) Within Scotland, the incidence of paracetamol-induced ALF has been estimated at 8.4 cases per annum per million population.(Newsome and others 2001b) The syndrome of ALF is not a single clinical entity, and may be precipitated by a wide variety of hepatic insults (**Table 1.1**).(Sass and Shakil 2005) These insults have a marked geographical and socioeconomic variation, with the most common aetiologies in Europe and North America being paracetamol and idiosyncratic drug reactions, whereas developing countries have a higher preponderance of acute viral aetiologies (**Table 1.2**). Specific insults also demonstrate geographical variation, with staggered accidental paracetamol overdoses predominating in the US, whereas single, intentional, overdoses are more common in the UK.(Larson and others 2005; Makin, Wendon, Williams 1995) The early identification, where possible, of the underlying aetiology of ALF is crucial since several causes of ALF such as paracetamol (*N*-acetyl cysteine (NAC)), *Amanita phalloides* poisoning (penicillin and silibinin), fulminant hepatitis B (lamivudine), herpes simplex virus (HSV) (acyclovir), and pregnancy (delivery) have specific treatments and the prognosis between different aetiologies varies considerably.(Heard 2008; Klein and others 1989; Kumar and others 2007; Miyake and others 2008; Montalbano, Slapak-Green, Neff 2005)

Aetiological category	Specific causes
Viral	HAV, HBV +/- HDV, HEV, HSV, Human herpes virus 6, CMV, EBV, VZV, parvovirus B19, yellow fever
Drug/ toxin induced (dose-dependent)	Paracetamol, *Amantina phalloides,* tetracyclines, *Bacillus cereus,* carbon tetrachloride,
Drug/ toxin-induced (idiosyncratic)	Halothane, anti-tuberculous therapy, sulphonamides, coamoxiclav, macrolides, valproate, NSAIDs, disulfiram, thalidomide, β-interferon, HAART, Ecstasy, cocaine, herbal remedies etc
Vascular	Ischaemic hepatitis, Budd-Chiari, right heart failure, venoocclusive disease
Metabolic	Wilson's disease , acute fatty liver of pregnancy, HELLP
Miscellaneous	Autoimmune hepatitis, malignant infiltration, sepsis, heat stroke
Others	Cryptogenic, primary graft non-function post-OLT

Table 1.1: Selected aetiologies of ALF

Abbreviations: HAV, hepatitis A virus; HBV, hepatitis B virus; HDV, hepatitis delta virus; HEV, hepatitis E virus; HSV, herpes simplex virus; CMV, cytomegalovirus; EBV, Epstein–Barr virus; VZV, Varicella zoster virus; HELLP, haemolysis, elevated liver enzymes and low platelets; HAART, Highly Active Anti-Retroviral Treatment.

Country	UK*	US	Canada	Scandinavia	France	Spain	Chile†	Australasia	Sudan	India
Reference	(Bernal and others 2009)	(Ostapowicz and others 2002)	(Tessier, Villeneuve, Villeneuve 2002)	(Brandsaeter and others 2002)	(Ichai and Samuel 2008)	(Escorsell and others 2007)	(Uribe and others 2003)	(Gow and others 2004)	(Mudawi and Yousif 2007)	(Khuroo and Kamili 2003)
No cases	310	308	81	315	363	267	27	80	37	180
Years	1994- 2004	1998-2001	1991- 1999	1990- 2001	1986-2006	1992-2000	1995-2003	1988-2001	2003-2004	1989- 1996
Paracetamol (%)	43	39	15	17	7	2	0	36	0	0
Non-paracetamol drug reactions (%)	8	13	12	10	21	14	7	6	8	0.6
Hepatotropic viruses (%)	7	12	30	12	33	37	37	14	27	68 (44 Hep E)
Indeterminate (%)	30	17	27	43	18	32	44	34	38	31
Other causes (%)	12	19	16	17	21	15	11	10	27	0

Table 1.2: Aetiologies of ALF by geographical location * Patients listed for OLT only † Paediatric patients only

1.4 Clinical features

The initial clinical features of ALF may be non-specific, and may include anorexia, fatigue, abdominal pain, jaundice, and fever before progressing to HE.(Shakil and others 2000) The 'type A' HE associated with ALF (types B and C HE are associated with portosystemic bypass and cirrhosis respectively) is graded from 1 to 4 on clinical features and neurological signs (**Table 1.3**).(Ferenci and others 2002) This grading is clinically robust and increasing grades of HE have a strong negative correlation with outcome. The pathogenesis of HE (and subsequent cerebral oedema) in ALF is incompletely understood, and differs from the encephalopathy seen in chronic liver disease, but hyperammonaemia, systemic inflammation, and loss of cerebral blood flow (CBF) autoregulation all appear to accelerate progression.(Bernal and others 2007a; Butterworth 2002; Rolando and others 2000) Ammonia, produced from glutamine by enterocytes, enters the systemic circulation via the portal vein, but is poorly cleared by the failing liver, and this is exacerbated by the coexistent renal failure, reduced hepatic urea synthesis, and impaired skeletal muscle function seen in ALF.(Wendon and Lee 2008) Astrocytes detoxify ammonia by converting it to glutamine which leads to osmotic swelling, and this cytotoxic process appears to be the main pathophysiological driver of cerebral oedema in ALF.(Gimson 1996; Haussinger and others 2000; Ranjan and others 2005) Hyponatraemia, frequently seen in patients with ALF, may potentiate this process, possibly via aquaporin-4.(Cordoba, Gottstein, Blei 1998; Rama Rao and Norenberg 2007) Additionally, cerebral hyperaemia, potentially mediated by pro-inflammatory cytokines, increases intracranial blood volume, further compromising cerebral perfusion.(Antoniades and others 2006; Wright and others 2007) Interestingly, case reports have directly implicated liver-derived toxins in this process.(Jalan and others 2002)

The evolution to grade III/IV HE is a grave prognostic sign as this group are at risk of intracranial hypertension, and subsequent brain herniation.(Clemmesen and others 1999) In addition, intracranial hypertension compromises cerebral perfusion pressure (CPP), defined as the difference between mean arterial pressure and intracranial pressure (ICP). A CPP >60 mmHg is considered crucial to maintain normal neurological functioning and periods >2 hours with a CPP <40 mmHg is considered by some centres to preclude liver transplantation.(Hoofnagle and others 1995) Clinical signs suggestive of increasing ICP include worsening of HE, systemic hypertension and bradycardia (Cushing reflex), altered

pupillary reflexes, and decerebrate rigidity. All of these clinical signs occur late in the clinical course, when therapeutic interventions may be ineffective, and this has led to the direct monitoring of ICP in patients with ALF.

HE Grade	Mental status	Neurological signs	EEG	GCS
I	Trivial lack of awareness; euphoria or anxiety; shortened attention span; impairment of addition/ subtraction	Slight tremor; apraxia; incoordination	Usually normal	15
II	Lethargy or apathy; disorientation for time; obvious personality change; inappropriate behaviour	Asterixis; ataxia; dysarthria	Generalised slowing	11-15
III	Somnolence to semi-stupor; responsive to stimuli; confused; gross disorientation; bizarre behaviour	Asterixis; ataxia	Abnormal	8-11
IV	Coma; unable to test mental state	Decerebration	Abnormal	<8

Table 1.3: Classification of HE (Amodio and others 2004)

Abbreviations: EEG, electroencephalogram; GCS, Glasgow Coma Scale.

1.4.1 Intracranial pressure monitoring

There remains some controversy over the use of ICP monitoring in ALF, due to the lack of consensus over treatment goals, the associated risks of bleeding and infection, and the lack of randomised trial data supporting improved survival. However, continuous monitoring permits rapid, targeted, treatment to be initiated and several groups now include ICP monitoring as part of their standard ALF protocol, particularly in potential OLT candidates.(Hoofnagle and others 1995; Keays, Alexander, Williams 1993) Intracerebral bleeding occurs in between 8-10% of cases, although fatal bleeds occur considerably less frequently.(Blei and others 1993; Vaquero and others 2005) Calculation of cerebral oxygen consumption using a jugular bulb catheter may provide additional information through continuous, indirect, assessment of cerebral blood flow.(Goetting and Preston 1990) This involves the retrograde passage of a fine-bore catheter into the jugular vein until the tip reaches the jugular bulb; venous saturations >85% (normal range 55-70%) representing a hyperaemic cerebral circulation.

1.4.2 Treatment for raised ICP

The goal of medical management of cerebral oedema should be to keep the ICP<20 mmHg and the CPP>70 mmHg, by reducing brain volume or cerebral blood flow (CBF). Basic manoeuvres include elevation of the head of the bed to no more than 30° and minimising painful stimuli including suctioning.(Davenport, Will, Davison 1990) Hyperventilation produces, at best, a transient restoration of cerebral blood flow autoregulation by lowering $PaCO_2$ and its prolonged use in ALF patients has been questioned.(Ede and others 1986) Established therapies for raised ICP include mannitol and barbituates such as thiopentone.(Canalese and others 1982; Forbes and others 1989) More limited evidence supports the use of hypertonic saline, propofol sedation, and indomethacin.(Murphy and others 2004; Tofteng and Larsen 2004) Unrandomised studies from Edinburgh have advocated the use of moderate hypothermia (32-33 °C) in advanced ALF as a means of reducing ICP prior to, and during, transplantation and this appears to offer a therapeutic option which targets many of the proposed triggers for elevated ICP in ALF.(Jalan and others 1999; Jalan and others 2003; Jalan and others 2004; Wendon and Lee 2008) Further studies are required to clarify the optimal extent and duration of hypothermia and to exclude a negative impact from hypothermia upon sepsis, coagulopathy, and cardiac stability.

1.5 Haemodynamic, respiratory, and renal instability

ALF is characterised by a hyperdynamic circulation, with markedly reduced systemic vascular resistance, increased cardiac output, and hypotension, which frequently necessitates vasopressor support in addition to fluid repletion.(Bihari and others 1985) As yet, the optimal fluid and vasopressor strategy remains uncertain, but most centres use crystalloid resuscitation followed by norepinephrine infusions to maintain adequate perfusion pressures and cerebral oxygenation, although this strategy may not significantly improve oxygen delivery.(Wendon and others 1992) Over-zealous norepinephrine use should be avoided since this may exacerbate cerebral hyperaemia due to the loss of CBF autoregulation in ALF.(Larsen and others 2000) Terlipressin, a vasopressin synthetic analogue, has been evaluated in several small studies which produced conflicting results regarding its effects upon CBF, ICP, and systemic haemodynamic parameters.(Eefsen and others 2007; Shawcross and others 2004)

Critical-illness related corticosteroid insufficiency (CIRCI) may exacerbate the haemodynamic instability seen in ALF, with a negative correlation between illness severity and the response to short synacthen testing in a cohort of 45 patients with acute liver injury.(Harry, Auzinger, Wendon 2002) Corticosteroid treatment of patients with low baseline cortisol levels ('hepatoadrenal syndrome') in a liver intensive care unit resulted in reduced vasopressor requirements and mortality, but it is worth noting that adrenal insufficiency was commoner in patients with chronic liver disease or post-OLT in this study than in the ALF patients.(Marik and others 2005) There is insufficient evidence at this stage to recommend routine treatment of CIRCI in liver patients, and the use of synacthen tests to diagnose CIRCI is not recommended.(Marik and others 2008)

Radiographic changes suggestive of pulmonary oedema occur in a high proportion of ALF cases, and the development of severe acute lung injury is associated with a poor prognosis.(Baudouin and others 1995; Trewby and others 1978) Treatment of ARDS using a protective ventilatory strategy is more problematic in ALF because increases in positive end expiratory pressure may exacerbate cerebral oedema and hepatic congestion.(Sass and Shakil 2005)

Renal failure in ALF is multifactorial, related to acute tubular necrosis, hypoperfusion, use of contrast agents and, in paracetamol-induced ALF, direct nephrotoxicity.(Blantz 1996) The Systemic Inflammatory Response Syndrome (SIRS) has recently been shown to predict renal dysfunction in non-paracetamol induced ALF.(Leithead and others 2009) Management should focus on prevention of renal failure through maintaining adequate systemic blood pressure, prompt identification and treatment of infections, and judicious use of contrast agents, since once established, the prognosis is considerably poorer. Continuous, rather than intermittent, methods of extracorporeal support are preferred to minimise circulatory and cerebral fluctuations.(Davenport, Will, Davidson 1993) Hypophosphataemia is frequent in ALF and may be linked to hepatic regeneration; persistently elevated phosphate levels suggest failure of this regenerative process and have been associated with a poorer prognosis in paracetamol-induced ALF.(Knochel 1989; Schmidt and Dalhoff 2002) Reduced hepatic glycogen stores and hyperinsulinaemia contribute to hypoglycaemia which complicates up to 40% of ALF cases, and continuous glucose administration is frequently required. Oral or enteral feeding is vital, but the markedly increased energy expenditure seen in ALF makes adequate nutritional support difficult to institute effectively in established liver failure.(Walsh and others 2000)

1.6 Coagulopathy

The coagulopathy of ALF is complex and remains poorly characterised, but is undoubtedly of prognostic significance.(Harrison and others 1990a; Izumi and others 1996; O'Grady and others 1989) ALF is characterised by prolongation of prothrombin time, quantitative and qualitative platelet dysfunction, and, in paracetamol-induced ALF, hypofibrinogenaemia and reductions in coagulation factors II, V, VII and X.(Kerr and others 2003) However, despite the severity of the coagulopathy, clinically significant spontaneous bleeding is relatively unusual in ALF, even during liver transplantation.(de Boer and others 2005; Munoz and others 2008) The defective production of procoagulant factors is compensated for in part by underproduction of anticoagulant proteins protein C, protein S, and antithrombin III whilst factor VIII production is upregulated, possibly in extrahepatic sites.(Hollestelle and others 2005; Lisman and Leebeek 2007) Plasminogen activator inhibitor-1 levels are grossly elevated in ALF suggesting hypofibrinolysis, and this was

supported by a recent murine study where heparin pre-treatment significantly reduced hepatic fibrin deposition following paracetamol poisoning and significantly attenuated paracetamol-induced hepatotoxicity.(Ganey and others 2007a; Pernambuco and others 1993) The bleeding risk in ALF has been overstated, and the prophylactic administration of large volumes of fresh frozen plasma (FFP) in ALF is unnecessary, interferes with prognostic scoring systems, and may worsen cerebral oedema or volume overload.(Caraceni and Van Thiel 1995; Stravitz 2008) Several pilot studies have suggested that recombinant factor VII may be superior to FFP for clinically significant bleeding in ALF, but further evaluation is required before this expensive treatment can be universally recommended.(Kalicinski and others 1999; Shami and others 2003) At present, FFP, platelet and cryoprecipitate infusions should be reserved for use in actively bleeding patients or prior to planned invasive procedures.(Fontana 2008)

1.7 Infection

The SIRS, and, when precipitated by infection, sepsis, have been shown to have a strong negative impact on HE progression, renal function, and mortality in ALF.(Anonymous 1992; Leithead and others 2009; Rolando and others 2000a) Bacteraemia is present in up to 80% of ALF patients who have enhanced susceptibility to infection due to the presence of indwelling lines and catheters, impaired complement and opsonisation function, and impaired innate immunity.(Wyke and others 1980) The majority of infections are caused by Gram-negative enteric organisms, staphylococci, and fungal infections.(Rolando and others 1990; Rolando and others 1991) The role of bacterial gut translocation, important in cirrhosis, is unclear at present, since prospective randomised trials of oral and enteral gut decontamination in ALF have failed to add additional benefit over parenteral antibiotic regimens alone.(Balzan and others 2007; Rolando and others 1993; Rolando and others 1996) Fungal infections are commonly under recognised and are particularly important in ALF patients who have received prolonged courses of antibiotics or have renal dysfunction.(Rolando and others 1991) Close surveillance for infection should be maintained in all ALF patients with frequent chest radiographs and cultures of blood, urine, and sputum, but prophylactic antibiotics should probably be reserved for those patients with high-grade encephalopathy, renal dysfunction, or those awaiting OLT.(Rolando and others 1993; Stravitz 2008)

1.8 Specific therapies in ALF

1.8.1 *N*-acetyl cysteine

When used early as an antidote after a single, intentional paracetamol overdose, NAC is extremely effective at replenishing hepatic glutathione stores and preventing severe *N*-acetyl-p-benzoquinone imine induced hepatotoxicity and liver failure.(Heard 2008) The evidence for NAC in patients with establishing hepatotoxicity or ALF is less robust, and is based upon a retrospective study from King's College Hospital and a small randomised controlled trial from the same centre.(Harrison and others 1990b; Keays and others 1991) Initial studies suggested that NAC improved oxygen delivery and consumption in ALF, but this assertion has subsequently been challenged.(Harrison and others 1991; Walsh and others 1998) Furthermore, the optimal duration of NAC therapy in these patients is unclear, since prolonged NAC therapy was recently shown to impair murine liver regeneration and worsen outcome following paracetamol poisoning.(Yang and others 2009) The benefit of NAC in non-paracetamol induced ALF is even less clear, and whilst a recent multicentre randomised controlled trial (RCT) has demonstrated marginally improved spontaneous survival in patients with Grade I-II HE, the intergroup differences and use of one-sided *p* values in this study are of concern.(Lee and others 2009)

1.8.2 Penicillin and silibinin

Mycetismus (mushroom poisoning), most commonly from *Amanita* genus mushrooms, is a medical emergency. Amantinin toxin, recycled via the enterohepatic circulation, interrupts hepatocyte messenger RNA synthesis and causes dose-dependent hepatotoxicity. Initial promising reports describing charcoal haemoperfusion in the treatment of *Amanita* poisoning have not been replicated.(Wauters, Rossel, Farquet 1978) Penicillin G (250 mg/kg/day) and silibinin (20-50 mg/kg/day) are effective when administered early in the course of the disease, but severe cases may require emergency OLT.(Broussard and others 2001; Klein and others 1989)

1.8.3 Lamivudine

Fulminant hepatitis B is frequently associated with delta virus coinfection.(Gomes-Gouvea and others 2009) Previous case reports utilised foscarnet in the treatment of fulminant

hepatitis B, but now increasing evidence supports the efficacy of lamivudine in this situation, even in pregnant patients.(Hedin and others 1987; Kumar and others 2007; Miyake and others 2008; Price and others 1986; Tillmann and others 2006)

1.8.4 Delivery

The syndrome of haemolysis, elevated liver enzymes, and low platelets (HELLP) and acute fatty liver of pregnancy (AFLP) are the pregnancy-related liver disorders most commonly associated with ALF, although preeclampsia can occasionally result in hepatic rupture and infarction. There is increasing evidence that preeclampsia, HELLP, and AFLP represent a spectrum of the same disease, with similar clinical and pathophysiological correlations including increased vascular tone and platelet aggregation.(Rahman and Wendon 2002) Foetal deficiency of the mitochondrial enzyme long-chain 3-hydroxyacyl-CoA dehydrogenase results in maternal accumulation of medium- and long-chain fatty acids, and similar mutations of the mitochondrial trifunction protein have been linked to both HELLP and AFLP.(Ibdah and others 1999; Treem and others 1994; Wilcken and others 1993) Preeclampsia, HELLP, and AFLP all carry significant risk of both maternal and foetal mortality, and severe cases should be managed in tertiary centres capable of dealing with the potential obstetric and hepatic complications. The mainstay of treatment of all three conditions is delivery, but close post-partum observations of both mother and infant are important to detect any haemorrhagic complications or continued deterioration of laboratory parameters.(Lee and Brady 2009; Pereira and others 1997)

1.8.5 Aciclovir

Whilst HSV has high seroprevalence amongst the general population, HSV-related hepatitis is a rare cause of ALF (1.4% in one 13-year cohort study) but is most commonly seen amongst immunosuppressed and pregnant patients.(Ichai and others 2005) The diagnosis of HSV-ALF is frequently made late, with a resultant high mortality rate. A high index of suspicion must be maintained to screen for potential cases amongst young, immunosuppressed or pregnant individuals presenting with transaminitis, since prompt treatment with intravenous acyclovir is effective in many cases.(Levitsky and others 2008; Montalbano, Slapak-Green, Neff 2005)

1.8.6 Plasmapheresis and D-penicillamine

Fulminant Wilson's disease represents a rare, but important cause of ALF, and the diagnosis can be intimated by the presence of a low alkaline phosphatase to bilirubin ratio, AST:ALT ratio >2.2 and haemolytic anaemia.(Korman and others 2008; Sallie and others 1992) Isolated cases report the successful use of plasmapheresis and chelation therapy in the treatment of fulminant Wilson's, although these remain a bridge to transplantation rather than definitive therapies.(Asfaha and others 2007; Rodriguez Farina and others 2003) Plasmapheresis appears to reduce arterial ammonia levels, and has occasionally been utilised in the treatment of aetiologies of ALF other than Wilson's disease, but the side-effects of treatment can be significant and no mortality benefit has been demonstrated to date.(Barshes and others 2005; Clemmesen and others 2001; Lepore and Martel 1970)

1.8.7 Other treatments

Other treatments such as insulin/glucagon, prostaglandin E2 and corticosteroids (even in fulminant autoimmune hepatitis), have failed to show significant benefit in ALF and are not recommended.(Anonymous 1979; Cattral and others 1994; Harrison and others 1991; Ichai and others 2007; Woolf and Redeker 1991)

1.8.8 Artificial and bioartificial liver devices

Liver assist devices have received much attention over recent years in the hope they can provide an effective 'bridge' to transplantation or recovery of liver function. The initial artificial liver support devices were essentially filters designed to remove toxins through haemodialysis or adsorption using charcoal and failed to show a survival benefit in ALF.(O'Grady and others 1988) The Molecular Absorbent and Recirculating System (MARS) system utilises a hollow fibre, double-sided, albumin-impregnated dialysis membrane to extract protein-bound toxins into the albumin dialysate. (Barshes and others 2005) Initial reports suggested improvements in both systemic and cerebral haemodynamic parameters and improvements in HE in patients with ALF and acute-on-chronic liver failure.(Ben Abraham and others 2001; McIntyre and others 2002; Schmidt and others 2003; Sorkine and others 2001) More advanced systems, such as Prometheus™, involve fractionated plasma separation and adsorption whilst bioartificial liver systems include living human or porcine hepatocytes incorporated in an extracorporeal circuit to add synthetic function to the process of detoxification. At least 12 RCTs of these devices have been performed and they have been

systematically reviewed twice; overall, these devices improve HE but have no mortality benefit in ALF, but may improve outcome in acute-on-chronic liver failure.(Kjaergard and others 2003; Liu and others 2004)

1.8.9 Liver transplantation

Although never subjected to a RCT, OLT has been recommended for the treatment of ALF since 1983.(Anonymous 1984) Emergency OLT for ALF now accounts for 5-12% of all liver transplantation activity and is the only treatment to date to substantially alter the mortality of the condition.(O'Grady 2005a) Patient survival following OLT for ALF is generally poorer than those transplanted for chronic liver failure, particularly in the setting of pre-transplant renal failure, but is of the order of 65-80% 1-year survival.(Barshes and others 2006; Farmer and others 2003; Lidofsky 1993) Alternatives to standard OLT are being refined, including living donor grafts and auxiliary liver transplantation (where part of the native liver is left *in situ* after partial liver transplantation in the hope that native liver regeneration can permit later cessation of immunosuppression).(Campsen and others 2008; Chenard-Neu and others 1996) Whilst a promising therapy for paracetamol-induced ALF, auxiliary transplantation fails to remove the diseased native liver leading to concerns over continuing haemodynamic and neurological instability. The limited timescales and the dramatic nature of the disease progression involved mean that the possibility of coercion of potential living donors in the setting of ALF is of significant ethical concern. Additionally, ALF patients are at particular risk of small for size syndrome and therefore the larger right lobe from the donor is usually transplanted, increasing the risk of donor morbidity or mortality.(Umeshita and others 2003)

1.9 Prognostication to guide OLT in ALF

The decision to super-urgently list an ALF patient for OLT is rarely easy- the inherent risks associated with delaying listing for OLT must be balanced against the potential for spontaneous recovery with medical therapy alone, the risks of surgery in the context of an acute critical illness, the scarcity of donor grafts and the requirement (except after auxiliary liver transplantation) for lifelong immunosuppression.(Chenard-Neu and others 1996) Furthermore, up to 60% of paracetamol-ALF patients meeting poor prognostic criteria are deemed unsuitable to undergo OLT due to coexistent psychiatric or medical conditions likely to preclude long-term graft and/or patient survival, such as resistant alcohol or drug

dependence or previous persistent treatment non-compliance.(Simpson and others 2009) Accurate prognostication in ALF is therefore vital, and many proposed mathematical, serological, radiological, and histological variables have been proposed, including the Model for End-Stage Liver Disease (MELD) score, which has improved organ allocation in chronic liver disease (**Table 1.4**).(Wiesner 2004; Zaman and others 2006) Major methodological flaws exist with many of these studies, which are often unblinded, retrospective, and prone to spectrum bias.(Ding and Buckley 2008) Furthermore, many authors equate liver transplantation with death, falsely elevating the positive predictive value of the test in question.(Bailey, Amre, Gaudreault 2003) In 1989, O'Grady and colleagues developed the 'King's College Criteria' from a retrospective cohort of 588 patients with grade II-IV HE admitted to that institution.(O'Grady and others 1989) The criteria recognise the prognostic importance of the aetiology of ALF, with separate criteria for paracetamol-ALF and other aetiologies (**Table 1.5**). These criteria are remarkable for their robustness and accuracy despite numerous attempts to improve on their diagnostic accuracy (**Table 1.4**). The paracetamol criteria have recently been modified by the addition of lactate, although several authors have questioned the overall benefit of this modification.(Bates and others 2007; Bernal and others 2002; Schmidt and Larsen 2006) The King's College Criteria have also been criticised for their low sensitivity and negative predictive value, particularly for non-paracetamol aetiologies.(Renner 2007)

Prognostic variable	Aetiology	Predictor of poor prognostic outcome	Sensitivity	Specificity	References
KCC	All	See table 1.5	69	92	(O'Grady and others 1989)
Clichy criteria	All	HE+ Factor V < 20% (age < 30 yr) or < 30% (age > 30 yr) Grade III-IV HE + Factor V<20%	- 86	- 76	(Bernuau and others 1986) (Izumi and others 1996)
Factor V; factor VIII/V ratio	Paracetamol	Factor VIII/V ratio>30; Factor V <10%	91 91	91 100	(Pereira and others 1992)
Phosphate	Paracetamol	PO_4^{3-}>1.2 mmol/l on day 2 or 3 post overdose	89	100	(Chung, Sitrin, Te 2003; Schmidt and Dalhoff 2002)
APACHE II	All	APACHE II >19	68	87	(Larson and others 2005)
Gc-globulin	All	Gc-globulin<100mg/L Paracetamol Non-paracetamol	 73 30	 68 100	(Schiodt and others 1996)
Lactate	Paracetamol	Admission arterial lactate>3.5 mmol/l or >3.0 mmol/l after fluid resuscitation	81	95	(Bernal and others 2002)

Continued overleaf

Prognostic variable	Aetiology	Predictor of poor prognostic outcome	Sensitivity	Specificity	References
α-fetoprotein	Paracetamol	AFP<3.9 μg/L 24 hours post peak ALT	100	74	(Schmidt and Dalhoff 2005)
MELD	Paracetamol	MELD>33 at onset of HE	60	69	(Schmidt and Larsen 2007)
	Non-paracetamol	MELD>32	76	67	(Dhiman and others 2007)

Table 1.4 (continued): Alternative prognostic variables suggested for use in ALF

Abbreviations: KCC, King's College Hospital poor prognostic criteria; MELD, Model for End-stage Liver Disease; HE, hepatic encephalopathy; APACHE II, Acute Physiology and Chronic Health Evaluation II; AFP, alpha fetoprotein.

<table>
<tr><th colspan="2">Non-paracetamol</th></tr>
<tr><td colspan="2">List for transplantation if:</td></tr>
<tr><td colspan="2">• PT> 100 seconds (INR>6.5) irrespective of HE grade

<u>Or any 3 out of 5 of the following:</u>

• Unfavourable aetiology: (Seronegative hepatitis, Wilson's disease, idiosyncratic drug reaction, halothane)
• Age <10 or >40 years
• Jaundice to encephalopathy interval > 7 days
• PT>50 seconds (INR>3.5)
• Bilirubin >300μmol/L</td></tr>
<tr><th>Paracetamol</th><th>Modified criteria</th></tr>
<tr><td rowspan="3">List for transplantation if:

• Arterial pH< 7.3

Or all 3 of the following occur within a 24-hr period:

• Grade III-IV HE
• PT>100 secs (INR>6.5)
• Serum creatinine>300 μmol/L</td><td>Strongly consider listing for OLT if:

• Arterial lactate>3.5 mmol/l after early fluid resuscitation</td></tr>
<tr><td>List for transplantation if:

• Arterial pH<7.3, or arterial lactate>3.0 mmol/l after adequate fluid resuscitation</td></tr>
<tr><td>List for transplantation if all 3 of the following occur within a 24-hr period:

• Grade III-IV HE
• PT>100 secs (INR>6.5)
• Serum creatinine>300 μmol/L</td></tr>
</table>

Table 1.5: The KCH poor prognostic criteria for paracetamol and non-paracetamol aetiologies of ALF as applied in the UK as transplant criteria.(Bernal and others 2002; O'Grady and others 1989)

1.10 Conclusions

ALF remains a truly challenging condition to manage, and requires early recognition and transfer of patients to specialist centres offering intensive multidisciplinary input in order to effectively manage the myriad complications of the syndrome, and, in some cases, offer liver transplantation. The challenges ahead are to further elucidate the pathophysiology behind liver cell death and the ensuing multiorgan failure of ALF, which in turn may help improve prognostic models. Further multicentre controlled trials of artificial and bioartificial liver support systems are needed before recommendations can be made regarding their clinical utility in ALF.

Chapter 2. Prognostic tests of paracetamol-induced acute liver failure: a systematic review and meta-analysis

2.1.1 Introduction

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Chapter 2. Prognostic tests of paracetamol-induced acute liver failure: a systematic review and meta-analysis

2.1 Introduction

Paracetamol toxicity remains the leading cause of ALF in the developed world, accounting for over 40% of cases in selected case series.(Bernal and others 2009; Ostapowicz and others 2002) Whilst the vast majority of patients recover spontaneously following paracetamol overdose, a small number develop severe acute liver injury, HE and consequently, ALF. Despite significant advances in supportive care, the only effective treatment for the condition remains emergency OLT. The decision to transplant a patient with paracetamol-induced ALF involves balancing the inherent risks associated with delaying listing for OLT against the potential for spontaneous recovery with medical therapy alone, the risks of surgery in the context of a rapidly evolving critical illness, the scarcity of donor grafts, and the requirement for lifelong immunosuppression. Furthermore, the psychosocial implications of paracetamol overdose cases are considerable, with over 30% of patients that fulfil transplant criteria unsuitable for OLT due to severe psychological illness, or coexistent chronic alcohol or drug dependency.(Simpson and others 2009) Accurate prognostication in ALF is therefore vital to utilise OLT effectively and prevent unnecessary transplantation, whilst procuring donor organs in a timely fashion for those most likely to benefit.

In 1989 O'Grady and colleagues developed the 'King's College Criteria' (KCC) in an attempt to determine which patients with paracetamol and non-paracetamol-induced ALF have a poor prognosis with medical therapy alone, and will therefore benefit most from OLT (**Table 1.5**).(O'Grady and others 1989) The original KCC for paracetamol-induced ALF were highly specific, but have been criticised for their relatively low negative predictive value (Renner 2007) and, since up to 26% of patients with paracetamol toxicity are medically unfit to undergo surgery at the point they fulfil the KCC,(Simpson and others 2009) modifications to these criteria now utilise arterial lactate (>3.5 mmol/L after early fluid resuscitation or >3.0 mmol/L after adequate fluid resuscitation) in an attempt to extend the time-window for acquisition of a suitable graft.(Bernal and others 2002)

Over recent years a plethora of alternative prognostic variables have been proposed, in an attempt to improve, or replace, the KCC (**see Table 1.4**). These criteria variously involve radiological,(Yamagishi and others 2005) histological, (Baeg and others 1988) serological,(Schiodt and others 2005; Schmidt and Dalhoff 2005) or mathematical (Cholongitas and others 2006a; Kremers and others 2004; Yantorno and others 2007) indices, but their assessment is complicated by the use of OLT; due to the inevitably imperfect nature of current listing criteria a small proportion of ALF patients are transplanted who would have spontaneously survived, invalidating further assessment of that particular patient. Studies evaluating the prognostic accuracy of a particular variable should therefore exclude transplanted patients from subsequent analysis.

The purpose of this systematic review was to critically evaluate existing prognostic criteria for predicting death without transplantation in paracetamol-induced ALF, to update previous studies in this area,(Bailey, Amre, Gaudreault 2003; Ding and Buckley 2008) and to examine the accuracy of the more recently described prognostic variables.

2.2 Methods

2.2.1 Search Strategy and Study Selection

A systematic review of the medical literature using MEDLINE (1950 to June 2009), EMBASE (1980 to June 2009), and CINAHL (1982 to June 2009) was performed to identify studies recruiting adult (>age 16 years) patients that evaluated prognostic markers of paracetamol-induced ALF. Potential studies were identified by combining the search terms *"acetaminophen"* and *"paracetamol"* (as both Medical Subject Headings (MeSH) and free text terms) using the set operator OR. Prognostic studies of ALF were identified, and combined using the set operator OR, by using the terms *"Kings adj3 College adj3 Criteria"*, *"Clichy$"*, *"APACHE$"*, *"lactate"*, *"Gc-globulin"*, *"alpha-fetoprotein"*, *"phosphate"* and *"MELD"* (all free text terms) and the McMaster expert search strategy for prognostic studies. These studies were combined using the set operator AND with papers evaluating studies on *"liver failure"*, *"hepatic encephalopathy"* or *"liver failure, acute"* (all MeSH). The search was limited to human studies without language restrictions. Abstracts of the studies identified by the initial search were evaluated for appropriateness to the study question by two independent reviewers (DC, KS) and all potentially relevant papers were obtained and evaluated in detail. The bibliographies of these studies and relevant review articles were screened to perform a recursive search of the literature, and abstract books of international liver conferences from the preceding 4 years were hand-searched for potentially relevant studies.

Selected studies were required to report mortality data on cohorts of patients admitted to hospital with acute severe liver injury or ALF secondary to paracetamol overdose (**see table 2.1 for study eligibility criteria**). Acute severe liver injury was defined as severe hepatotoxicity (serum alanine aminotransferase (ALT) levels>1000 U/mL and co-existent coagulopathy), whilst ALF required the additional presence of HE, in patients without pre-existing liver disease. Unselected cohorts of patients with mixed etiologies of acute liver injury were eligible for inclusion if separate data for paracetamol overdose patients were available. Case control studies and studies comparing ALF patients with chronic liver disease patients were excluded due to potential bias in favour of the prognostic variable in question,(Altman 2001) as were studies where transplantation and death were combined as a single end-point. Studies were required to include more than 25 patients with paracetamol-induced acute liver injury, since the majority of studies in this area include the KCC as a

prognostic variable in addition to the main variable studied, and smaller studies may invalidate analysis.(Perel and others 2006) All potential articles were assessed independently by two researchers according to eligibility criteria, which were defined prospectively, and disagreements resolved by consensus.

- Adult patients (aged > 16 years) presenting with acute severe liver injury or ALF attributed to paracetamol hepatotoxicity
- Cohort studies
- Included >25 patients with paracetamol hepatotoxicity
- Mortality data recorded
- Data for death and liver transplantation considered separately

Table 2.1 Eligibility criteria for included studies

2.2.2 Data extraction and quality assessment

Data were extracted using predesigned forms by two separate reviewers (DC and KJS) on to a Microsoft Excel spreadsheet (Microsoft Corp, Redmond, WA, USA), and any discrepancies were resolved by consensus. The following data were extracted for each study: setting, country and geographical region, year(s) conducted, retrospective or prospective design, inclusion criteria, definitions of paracetamol-induced liver injury, definitions of ALF, total number of subjects included, total number of subjects with paracetamol-induced ALF or acute liver injury, total number of subjects with paracetamol-induced ALF transplanted, duration of follow-up, prognostic score used, timing of application of the prognostic score, and outcome definitions. Study quality was assessed semi-quantitatively according to six potential sources of bias that can be encountered in studies of prognostic variables (Hayden, Côté, Bombardier 2006) and a grade (poor, moderate, good or excellent) allocated (**Table 2.2**).

Study population	Study population adequately represents the population of interest; source population adequately described; sampling timeframe, place and period of recruitment described; inclusion and exclusion criteria described
Follow-up	Adequate study completion rate; loss to follow-up not associated with key characteristics; reasons for loss to follow-up described; no important differences between participants completing/not completing study
Prognostic factor	Prognostic measure clearly defined; continuous data reported and any cut-points described and appropriate; method and setting of measurement of prognostic factor identical for all patients; adequate proportion of study population has prognostic factor measured
Outcome measurement	Clear definition of outcome measure of interest described and recorded, including duration of follow-up; outcome measure valid; outcome measure identical
Confounding	All important confounders measured and accounted for at all stages of study design, performance and analysis
Analysis	Sufficient presentation of data to permit assessment of analytical analysis; appropriate model-building, study design etc.; no selective reporting of results

Table 2.2 Quality assessment of included studies (adapted from Hayden 2006 (Hayden, Côté, Bombardier 2006))

2.2.3 Data synthesis and analysis

Prognostic tests evaluating the outcomes of survival without transplantation or death without transplantation following paracetamol-induced liver injury were assessed individually. From each study cohort the total number of subjects dying without OLT, total number of subjects surviving without OLT, and total numbers of subjects fulfilling or not fulfilling the prognostic variable in question were extracted and 2x2 contingency tables constructed. The sensitivity, specificity, and diagnostic odds ratio (DOR) with their 95% confidence intervals (CI) were calculated for each test using Microsoft Excel (Microsoft Corp, Redmond, Washington, USA) and checked using Meta-DiSc version 1.4 (Universidad Complutense, Madrid, Spain). The value of a DOR ranges from zero to infinity, with higher values indicating better discriminatory test performance, and is calculated from the following formula: sensitivity/(1- sensitivity) / (1- specificity)/specificity.(Glas and others 2003) Where required, a zero cell correction of 0.5 was added to all cells to prevent computational problems arising where proportions were equal to zero.(Dinnes and others 2005) Where two or more studies analysed a particular prognostic marker, data were pooled using a random effects model and the pooled DOR calculated. Heterogeneity between studies was assessed using the I^2 statistic with a cut off of 50%, and the χ^2 test with a P value < 0.10, used to define a statistically significant degree of heterogeneity. We explored study setting and study design as potential reasons for heterogeneity.(Higgins and others 2003) These are exploratory analyses only, and the results should therefore be interpreted with caution.

2.3 Results

The search strategy identified 6507 studies of which 104 were potentially eligible for inclusion and were retrieved for further analysis (**Figure 2.1**). Of these, 14 were considered eligible for inclusion,(Anand, Nightingale, Neuberger 1997; Bates and others 2007; Bernal and others 1998; Bernal and others 2002; Bernal and Wendon 2003; Bernal and others 2007; Izumi and others 1996; Larson and others 2005; Mitchell and others 1998; O'Grady and others 1989; O'Grady and others 1991; Schmidt and Dalhoff 2005; Schmidt and Larsen 2006; Zaman and others 2006) including two studies published in abstract form only.(Bates and others 2007; Bernal and others 2007) Characteristics of included studies are provided in **Table 2.3**. Cohen's kappa test for inter-observer agreement was excellent at 0.86 (95% CI 0.75-0.98).

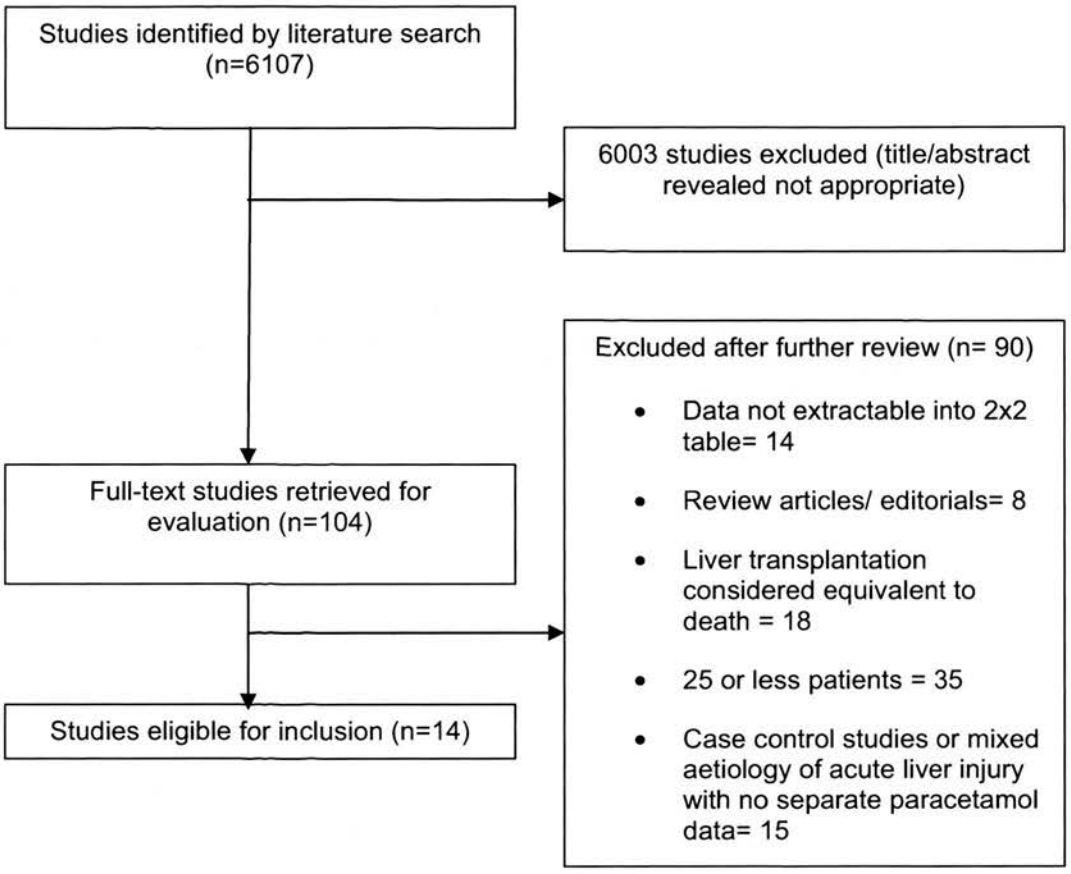

Figure 2.1 Flow diagram of assessment of studies identified in the systematic review

Study	Country	Liver Unit	Study period	Inclusion criteria	Prognostic test(s) evaluated	No paracetamol patients included	Retrospective/ prospective cohort	Retrospective/ prospective test development/ evaluation	Study quality
O'Grady 1989	UK	KCH	1973-1985; 1986-7	FHF as per Trey & Davidson with Grade III-IV HE	pH<7.3 Concurrent PT>100 seconds, serum creatinine> 300 mmol/L, Grade III/IV HE	121 99	Both	Prospective	Moderate
O'Grady 1991	UK	KCH	1988-1990	Severe liver damage	KCC as per O'Grady 1989	60	Prospective	Prospective	Moderate
Izumi 1996	UK	KCH	Not stated	FHF as per Trey& Davidson	KCC as per O'Grady 1989 Factor V ratio <20% Factor V ratio <10%	} 81	Prospective	Prospective (partially)	Poor
Anand 1997	UK	Birming-ham	1990-1994	FHF as per Trey & Davidson (Trey and Davidson 1970)	pH<7.3 Concurrent PT>100 seconds, serum creatinine> 300 mmol/L, Grade III/IV HE	72 89	Retrospective	Prospective	Poor
Bernal 1998	UK	KCH	1990-1996	Severe hepatotoxicity (KCC); Death whilst not meeting KCC (APACHE III)	KCC as per O'Grady 1989 Adapted APACHE III	504 56	Retrospective	Prospective (KCC) Retrospective (APACHE III)	Poor

Table 2.3 Characteristics of included studies (continued overleaf)

Study	Country	Liver Unit	Study period	Inclusion criteria	Prognostic test(s) evaluated	No paracetamol patients included	Retrospective/ prospective cohort	Retrospective/ prospective test development/ evaluation	Study quality
Mitchell 1998	UK	KCH	1993-1994	Coagulopathy + recent history paracetamol ingestion	KCC as per O'Grady(O'Grady and others 1989) APACHE II>15 at 24 hr post-admission APACHE II>15	} 94	Prospective	Prospective (KCC) Retrospective (APACHE II)	Good
Bernal 2002	UK	KCH	1998-1999 (learning set) 1999-2000 (validation set)	Severe paracetamol-induced hepatotoxicity	KCC as per O'Grady 1989 Arterial lactate>3.5 mmol/L at 4 hours Arterial lactate>3.0 mmol/L at 12 hours KCC + combination of lactate criteria	99 97 85 85	Both	Prospective	Moderate
Bernal 2003	UK	KCH	1998-2000	Acute severe hepatotoxicity	KCC as per O'Grady 1989 Phosphate >1.2 mmol/L on admission day +1 or 2	} 170	Retrospective	Prospective	Moderate
Larson 2005	USA	22 academic centres	1998-2003	INR>1.4; HE; jaundice to HE interval<26 weeks	KCC as per O'Grady 1989	252	Prospective	Prospective	Moderate
Schmidt 2005	Denmark	Copenhagen	1999-2002	Peak ALT>1000U/L	KCC as per O'Grady 1989 AFP <3.9 at D1 post peak ALT AFP <3.9 & INR>2.4 at D1 post peak ALT	234 188 188	Prospective	Prospective (KCC) Retrospective (AFP)	Moderate

Table 2.3 (continued) Characteristics of included studies (continued overleaf)

Study	Country	Liver Unit	Study period	Inclusion criteria	Prognostic test(s) evaluated	No paracetamol patients included	Retrospective/ prospective cohort	Retrospective/ prospective test development/ evaluation	Study quality
Schmidt 2006	Denmark	Copenhagen	1999-2004	Severe paracetamol-induced FHF	KCC as per O'Grady 1989 Arterial lactate Modified KCC SOFA score >8 at admission/ >12 at onset of Grade III/IV HE SIRS at admission/at onset of Grade III/IV HE	95 91 91 95 95	Prospective	Retrospective	Moderate
Zaman 2006	Ireland	Dublin	1994-2005	Paracetamol-induced ALF (jaundice to HE <8 weeks) or rapid↑ bilirubin/ INR/ renal impairment/ hypoglycaemia if no HE	KCC as per O'Grady 1989 MELD >30	} 60	Retrospective	Prospective	Poor
Bates 2007	UK	Edinburgh	2004-2007	Acute severe liver injury	KCC as per O'Grady 1989 Lactate modifications to KCC as per Bernal 2002	69 32	Retrospective	Prospective	Poor
Bernal 2007	UK	KCH	Not stated	Acute liver failure	IL-6 within 24 hours of admission	31	Prospective	Retrospective	Poor

Table 2.3 Characteristics of included studies

PT, prothrombin time; FHF, fulminant hepatic failure

The eligible studies evaluated a total of 1960 patients with paracetamol-induced acute liver injury or ALF. Three (Izumi and others 1996; Mitchell and others 1998; Schmidt and Dalhoff 2005) studies had complete temporal overlap with other studies from the same unit; however, these studies were included for their evaluation of unique prognostic markers separate to the KCC. There was only one multicentre study.(Larson and others 2005) Five (Anand, Nightingale, Neuberger 1997; Bates and others 2007; Bernal and others 1998; Bernal and Wendon 2003; Zaman and others 2006) studies evaluated patient cohorts retrospectively, whilst five (Bernal and others 1998; Bernal and others 2007; Mitchell and others 1998; Schmidt and Dalhoff 2005; Schmidt and Larsen 2006) studies developed prognostic test thresholds retrospectively (usually from receiver operator characteristic (ROC) curve analysis). No studies blinded observers to patient outcome or other potentially confounding prognostic data; and only two (Bernal and others 2002; O'Grady and others 1989) validated their prognostic marker in a separate cohort. Consequently, six (Anand, Nightingale, Neuberger 1997; Bates and others 2007; Bernal and others 1998; Bernal and others 2007; Izumi and others 1996; Zaman and others 2006) of the 14 studies were graded as poor and seven (Bernal and others 2002; Bernal and Wendon 2003; Larson and others 2005; O'Grady and others 1989; O'Grady and others 1991; Schmidt and Dalhoff 2005; Schmidt and Larsen 2006) as moderate quality (**Table 2.3**). The 14 eligible studies analysed a total of 22 different prognostic markers or variations thereof. The sensitivity, specificity, and DORs of these are reported in **Table 2.4**.

Prognostic test	Study	N/deaths	Test +ve/ deaths	Sensitivity (95% CIs)	Specificity (95% CIs)	Diagnostic Odds Ratio (95% CIs)	Heterogeneity	
							χ^2	I^2
pH<7.3	O'Grady 1989	121/43	22/21	48.8 (33.3-65.5)	98.7 (93.1-100)	73.5 (9.4-577.4)		
	Anand 1997(Anand, Nightingale, Neuberger 1997)	72/39	31/24	61.5 (44.6-76.6)	78.8 (61.1-91.0)	5.9 (2.1-17.1)		
	POOLED			**54.9 (43.5-65.9)**	**92.8 (86.3-96.8)**	**18.0 (1.1-229.6)**	4.91 p=0.027	80%
Concurrent PT>100s, serum creatinine>300 mmol/L, Grade III/IV HE	O'Grady 1989	99/22	15/10	45.5 (24.4-67.8)	93.5 (85.5-97.9)	12.0 (3.5-41.3)		
	Anand 1997	89/45	24/19	42.2 (27.7-57.9)	88.6 (75.4-96.2)	5.7 (1.9-17.2)		
	POOLED			**43.3 (31.2-56.0)**	**91.7 (85.3-96.0)**	**7.9 (3.5-18.1)**	0.8 p=0.374	0%
KCC (combined)†	O'Grady 1989	220/65	37/31	47.7 (35.1-60.5)	96.1 (91.8-98.6)	22.6 (8.8-58.6)		
	O'Grady 1991	60/26	23/19	73.1 (52.2-88.4)	88.2 (72.6-96.7)	20.4 (5.2-79.0)		
	Bernal 1998	504/99	80/71	71.7 (61.8-80.3)	97.8 (95.8-99.0)	111.6 (50.5-246.4)		
	Bernal 2002	99/21	20/16	76.2 (52.8-91.8)	94.9 (87.4-98.6)	59.2 (14.3-245.3)		
	POOLED (KCH)			**65.9 (59.0-72.3)**	**96.1 (94.4-97.5)**	**43.9 (17.6-109.3)**	8.4 p=0.038	64%
	Larson 2005 (at admission)	252/74	34/19	25.7 (16.2-37.2)	91.6 (86.5-95.2)	3.8 (1.8-7.9)		
	Schmidt 2006	95/48	36/27	77.1 (62.7-88.0)	83.0 (69.2-92.4)	16.4 (5.9-45.3)		
	Zaman 2006	60/29	21/21	72.4 (52.8-87.3)	100.0 (88.8-100.0)	159.4 (8.7-2908.9)		
	Bates 2007	69/17	18/15	88.2 (63.6-98.5)	94.2 (84.1-98.8)	122.5 (18.7-803.1)		
	POOLED (non-KCH)			**48.8 (41.0-56.6)**	**91.2 (87.5-94.1)**	**16.5 (3.5-77.8)**	17.0 p=0.001	82%
	POOLED (overall)			**58.2 (53.1-63.3)**	**94.6 (93.0-95.9)**	**27.7 (9.2-83.5)**	53.0 p<0.001	87%

Table 2.4 (continued overleaf) Sensitivity, specificity, and diagnostic odds ratios of individual prognostic markers.

† Anand 1997 excluded as 2x2 table not reconstructable; Mitchell 1998, Bernal 2003, and Schmidt 2005 excluded due to complete temporal overlap with previous studies from same unit

Prognostic test	Study	N/deaths	Test +ve/ deaths	Sensitivity (95% CIs)	Specificity (95% CIs)	Diagnostic Odds Ratio (95% CIs)	Heterogeneity	
							χ^2	I^2
Arterial lactate >3.5 at admission	Bernal 2002 Schmidt 2006 Bates 2007 **POOLED**	97/21 91/46 69/17	18/14 61/39 37/15	66.7 (43.0-85.4) 84.8 (71.1-93.7) 88.2 (63.6-98.5) **81.0 (70.9-88.7)**	94.7 (87.1-98.6) 51.1 (35.8-66.3) 57.7 (43.2-71.3) **72.3 (64.9-78.8)**	36.0 (9.3-139.6) 5.8 (2.2-15.8) 10.2 (2.1-49.4) **12.2 (4.0-37.4)**	4.54 p=0.103	56%
Arterial lactate >3.0 following resuscitation	Bernal 2002 Schmidt 2006* Bates 2007*at onset of Grade III/IV HE **POOLED**	85/21 91/46 32/12	18/16 55/36 20/12	76.2 (52.8-91.8) 78.3 (63.6-89.1) 100.0 (73.5-100.0) **81.0 (70.6-89.0)**	96.9 (89.2-99.6) 57.8 (42.2-72.3) 60.0 (36.1-80.9) **77.5 (69.3-84.4)**	99.2 (17.6-559.3) 4.9 (2.0-12.3) 36.8 (1.9-708.0) **22.8 (2.5-210.0)**	9.83 p=0.007	80%
Arterial lactate >4.0 at admission	Schmidt 2006	91/46	51/34	73.9 (58.9-85.7)	62.2 (46.5-76.2)	4.7 (1.9-11.4)	-	-
Arterial lactate >4.0 at onset of Grade III/IV HE			40/31	67.4 (52.0-80.5)	80.0 (65.4-90.4)	8.3 (3.2-21.5)		
KCC + arterial lactate >3.0 following resuscitation	Bernal 2002(Bernal and others 2002) Schmidt 2006 **POOLED**	85/21 91/46	24/19 69/42	90.5 (69.6-98.8) 91.3 (79.2-97.6) **91.0 (81.5-96.6)**	92.2 (82.7-97.4) 40.0 (25.7-55.7) **70.6 (61.2-79.0)**	112.1 (20.1-625.7) 7.0 (2.1-22.9) **26.1 (1.7-393.7)**	8.83 p=0.003	89%
Factor V ratio <20%	Izumi 1996	81/35	69/34	97.1 (85.1-99.9)	23.9 (12.6-38.8)	10.7 (1.3-87.3)	-	-
Factor V ratio <10%			51/29	63.0 (47.6-76.8)	37.1 (21.5-55.1)	5.3 (1.8-15.1)		
Adapted APACHE III	Bernal 1998	56/28	19/16	57.1 (37.2-75.5)	89.3 (71.8-97.7)	11.1 (2.7-45.6)	-	-

Table 2.4 (continued overleaf) Sensitivity, specificity, and diagnostic odds ratios of individual prognostic markers.

Prognostic test	Study	N/deaths	Test +ve/ deaths	Sensitivity (95% CIs)	Specificity (95% CIs)	Diagnostic Odds Ratio (95% CIs)	Heterogeneity	
							χ^2	I^2
APACHE II>15 at 24 hr post-admission	Mitchell 1998	94/14	13/11	78.6 (49.2-95.3)	97.5 (91.3-99.7)	143.0 (21.4 -953.5)	-	-
APACHE II>15 within 5 days of admission			23/13	92.9 (66.1-99.8)	87.5 (78.2-93.8)	91.0 (10.7-772.8)		
Phosphate >1.2 mmol/L on admission day +1 or 2	Bernal 2003	170/52	55/42	80.8 (67.5-90.4)	89.0 (81.9-94.0)	33.9 (13.8-83.3)	-	-
AFP <3.9 at D1 post peak ALT	Schmidt 2005	188/33	54/33	100.0 (89.4-100.0)	73.6 (65.9-80.3)	184.9 (11.1-3085.6)	-	-
AFP <3.9 & INR>2.4 at D1 post peak ALT			74/33	100.0 (89.4-100.0)	86.5 (80.0-91.4)	419.1 (24.8-7097.5)		
SOFA score >8 at admission	Schmidt 2006	95/48	48/32	66.7 (51.6-79.6)	66.0 (50.7-79.1)	3.9 (1.7-9.1)	-	-
SOFA score >12 at onset of Grade III/IV HE			54/39	81.3 (67.4-91.1)	68.1 (52.9-80.9)	9.2 (3.6-23.9)		
SIRS at admission	Schmidt 2006	95/48	52/33	68.8 (53.7-81.3)	59.6 (44.3-73.6)	3.2 (1.4-7.5)	-	-
SIRS at onset of Grade III/IV HE			41/34	70.8 (55.9-83.0)	85.1 (71.7-93.8)	13.9 (5.0-38.3)		
MELD >30	Zaman 2006	60/29	38/28	96.6 (82.2-99.9)	67.7 (48.6-83.3)	58.8 (7.0-495.8)	-	-
IL-6 within 24 hours of admission	Bernal 2007	31/8	7/6	75.0 (34.9-96.8)	95.7 (78.1-99.9)	66.0 (5.1-857.7)	-	-

Table 2.4 Sensitivity, specificity, and diagnostic odds ratios of individual prognostic markers.

2.3.1 King's College Criteria

A total of 13 studies evaluated the original KCC for paracetamol-induced ALF, either as separate elements (arterial pH<7.3 or concurrent grade III/IV HE, serum creatinine>300μmol/L and prothrombin time (PT)>100 seconds) (Anand, Nightingale, Neuberger 1997; O'Grady and others 1989) or as a whole, (Bates and others 2007; Bernal and others 1998; Bernal and others 2002; Bernal and Wendon 2003; Izumi and others 1996; Larson and others 2005; Mitchell and others 1998; O'Grady and others 1991; Schmidt and Dalhoff 2005; Schmidt and Larsen 2006; Zaman and others 2006) including a total of 1929 patients. One study (Larson and others 2005) evaluated the KCC only on admission. After exclusion of studies with complete temporal overlap with other studies,(Izumi and others 1996; Mitchell and others 1998; Schmidt and Dalhoff 2005) pooled specificity of the KCC was high, at 94.6% (95% CI 93.0-95.9), but the pooled sensitivity was relatively poor at 58.2% (95% CI 53.1-63.3). The pooled DOR for the KCC was 27.7 (95% CI 9.2-83.5) (**Table 2.4**). The summary ROC curve is shown in **Figure 2.2**. The area under the curve (AUC) was calculated as 0.91 (95% CI 0.79-0.99), suggesting good performance of the KCC overall.(Jones and Athanasiou 2005) The accuracy of the KCC improved when studies originating from KCH were analysed separately from those outside KCH (DOR 43.9 (95% CI 17.6-109.3) for KCH-based studies vs. DOR 16.5 (95% CI 3.5-77.8) for non-KCH-based studies). Statistically significant heterogeneity (I^2=87%) existed between individual study results, though this fell to 79% after exclusion of the single multi-centre study,(Larson and others 2005) and to 64% after exclusion of studies from outside KCH.

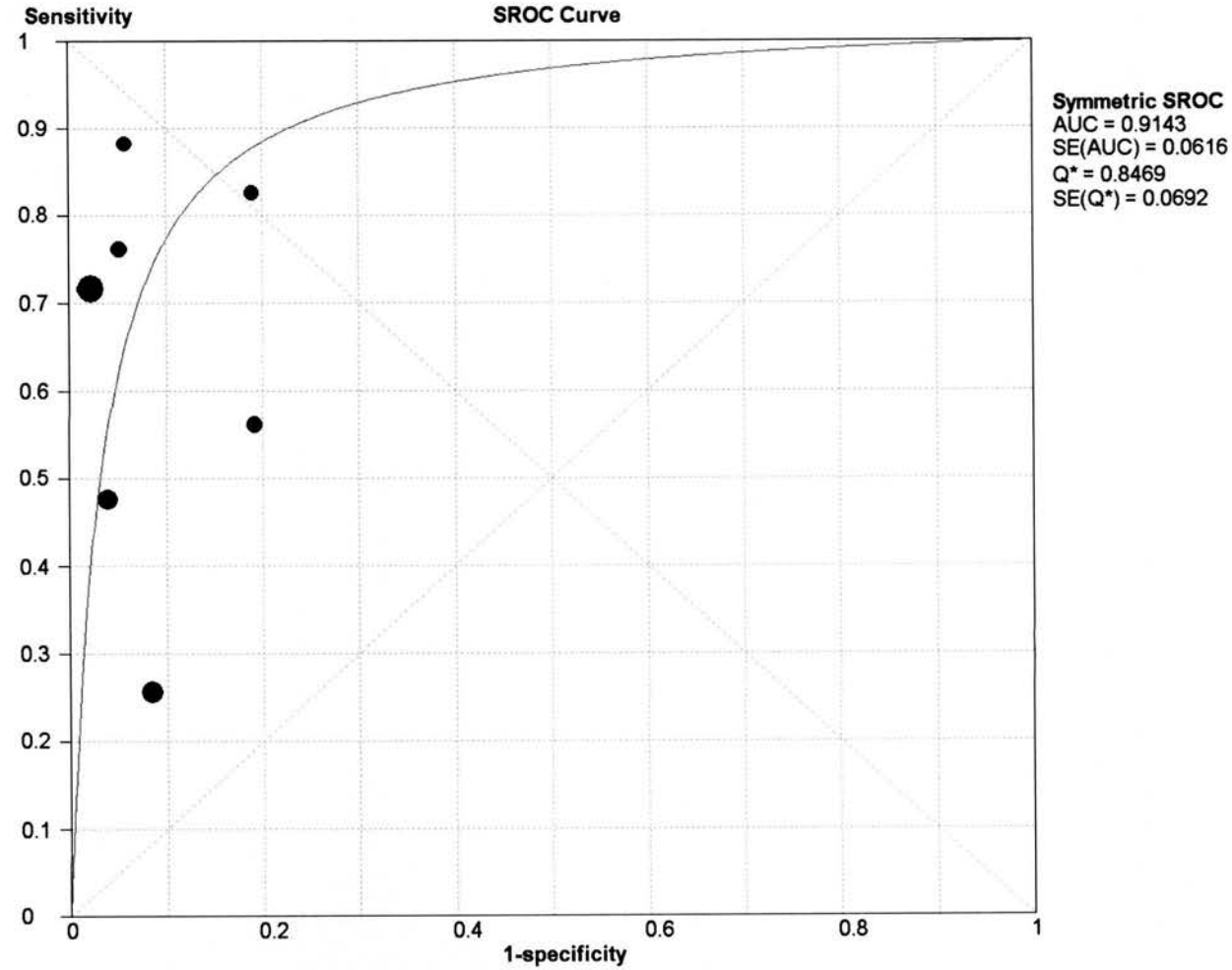

Figure 2.2 Summary receiver operator characteristic (SROC) of studies evaluating the original KCC

2.3.2 Lactate modifications to the KCC

The original study (Bernal and others 2002) that evaluated arterial lactate in the prognosis of paracetamol-induced ALF reported similar specificity, but improved sensitivity, when compared with the original KCC. However, two (Bates and others 2007; Schmidt and Larsen 2006) subsequent studies evaluating arterial lactate alone failed to replicate this high prognostic accuracy (**Table 2.4**), with pooled DORs of 12.2 (95% CI 4.0-37.4) and 22.8 (95% CI 2.5-210.0) for the early and post-resuscitation lactate values respectively. This was mainly due to the reduced specificity seen in the two studies from outside KCH. Combination of the KCC with a post-resuscitation lactate value >3.0 offered higher prognostic accuracy in the original lactate study (Bernal and others 2002) but this was not replicated in a subsequent evaluation.(Schmidt and Larsen 2006)

2.3.3 Other prognostic markers

Several other markers, all evaluated in single studies, appeared to offer improved prognostic accuracy when compared with the KCC, with low serum alpha-fetoprotein (AFP) (DOR 419.1),(Schmidt and Dalhoff 2005) 24-hour post-admission Acute Physiology and Chronic Health Evaluation II (APACHE II) score (DOR 143.0),(Mitchell and others 1998) serum interleukin-6 (IL-6) levels (DOR 66.0),(Bernal and others 2007) Model for End-Stage Liver Disease (MELD) score (DOR 58.8),(Zaman and others 2006) and serum phosphate (DOR 33.9) (Bernal and Wendon 2003) all outperforming the KCC (**Table 2.4**). However, further studies would be required to replicate these findings to confirm or refute whether these other markers are indeed superior to the KCC.

2.4 Discussion

This systematic review and meta-analysis has demonstrated that the original KCC for paracetamol-induced ALF have high pooled specificity (94.6%), but low pooled sensitivity (58.2%) in determining prognosis in patients with paracetamol-induced ALF. Additionally, the benefit of the arterial lactate modifications to the KCC (Bernal and others 2002) is questionable according to these data. Other proposed prognostic markers, in particular AFP, APACHE II scores, and serum IL-6 levels, showed encouraging prognostic accuracy but were only evaluated in single studies of variable quality.

This review is limited by the quality of the included studies, which had significant heterogeneity and were generally of poor or moderate quality, with only two (Bernal and others 2002; O'Grady and others 1989) studies validating their prognostic model prospectively in a separate cohort. ALF is a rare syndrome and, as a result, many studies were small and retrospective in nature. Continuous variables were frequently analysed retrospectively using cut-off values designed to maximise AUC values, a method which assumes constant risk amongst the 'high' and 'low' risk groups.(Perel and others 2006) Several studies attempted to evaluate multiple prognostic markers retrospectively using the same cohort, an approach which increases the risk of obtaining a statistically significant result by chance. Another potentially confounding issue is the lack of international standardisation of laboratory variables, such as prothrombin time (PT) and creatinine, and changes to assay reagents over the time course of these studies.(Newsome and others 2001a) We also appreciate that exclusion of studies where transplantation and death were deemed equivalent may have introduced spectrum bias, since prognostic tests are usually applied in settings where OLT is available. This problem is difficult to circumvent given that emergency OLT will never be subjected to a clinical trial, but, given the reduced quality-of-life seen following transplantation for paracetamol-induced ALF, the accumulated risks of immunosuppression, and the scarcity of liver donors,(Ding and Buckley 2008) accurate calculation of the specificity of each prognostic test is vital in order to minimise inappropriate transplantation.

This study expands upon a recent systematic review (Ding and Buckley 2008) evaluating the KCC for paracetamol-induced ALF by including additional prognostic markers. The former

study found spectrum bias in studies originating from KCH, with increased spontaneous survival amongst patients listed for transplantation, but not receiving a graft, compared with those meeting criteria but never listed, suggesting that a 'healthier' cohort may be preferentially transplanted in KCH. The current data confirm the increased prognostic accuracy of the original KCC in studies originating from KCH compared with studies from other units, with a higher pooled DOR in KCH-based studies compared with that from studies conducted in other units. This raises further questions regarding the overall generalisability of the KCC to patients with paracetamol-induced ALF treated outside KCH.

The heterogeneity of the included studies evaluating the KCC was partly due to a multicentre US study (Larson and others 2005) which explicitly applied the KCC solely at the time of admission, rather than dynamically throughout admission as originally intended.(O'Grady and others 1989; O'Grady 2007) Whilst early and accurate prognostication in ALF is vital in order to permit timely listing for OLT, application of the KCC solely at admission reduces the time available for the disease to evolve and may introduce spectrum bias. Given that the median time taken to fulfil the KCC following admission to a tertiary liver transplant unit is 12 hours,(Bernal and others 2002) application of the KCC solely at admission may explain the low sensitivity of the criteria in the US study, whilst concerns have also been expressed about the prophylactic use of fresh frozen plasma (FFP) in this US study and the potential confounding effects upon PT.(O'Grady 2005b) Furthermore, 48% of cases resulted from unintentional overdoses in this US study, a type of overdose seen less frequently in the UK.(Makin and Williams 2000) It may be that repeated ingestion of supratherapeutic doses of paracetamol over a protracted time course disrupts liver function subacutely, so that patients are less likely to develop the profound acidosis or concurrent severe coagulopathy, renal dysfunction, and encephalopathy required to fulfil the KCC, but have at least as poor a prognosis as an intentional overdose at a single time point. Future prognostic scoring systems may therefore need to take the pattern of paracetamol overdose into account in addition to traditional biochemical parameters. This concept is further explored in **chapter 3**.

The value of the addition of post-resuscitation arterial lactate to the KCC (Bernal and others 2002) is questioned by this study. Only two additional (Bates and others 2007; Schmidt and Larsen 2006) studies, one of which was reported in abstract-form, that reported this

modification fulfilled eligibility criteria for inclusion, but these studies suggest little benefit from this. Furthermore, the reduced specificity of the lactate criteria undermines the traditional benefit of the KCC in 'ruling in' a hopeless prognosis. The presence of systemic inflammation, with or without sepsis, is increasingly recognised as important in ALF,(Rolando and others 2000b) but hyperlactataemia can result from numerous other organ sources and therefore, perhaps not surprisingly, arterial lactate is a relatively non-specific prognostic indicator in paracetamol-induced ALF. Within critical care settings, the use of APACHE II and Sequential Organ Failure Assessment (SOFA) scores to monitor organ dysfunction have greater recognition than the KCC, and are attractive as early prognostic markers in ALF.(Cholongitas and others 2006b) APACHE II, in particular, showed encouraging prognostic accuracy but was only evaluated in a single eligible study.(Mitchell and others 1998) One (Larson and others 2005) additional study evaluating APACHE II scores was excluded from this particular analysis as the prognostic scoring reported in the study could not be reconstructed into a 2x2 table; this study is also notable for the atypical nature of the patient cohort as outlined above. Given that APACHE III, SIRS, and SOFA scores all performed less well than the KCC (albeit in single studies), the role of these markers may be to permit earlier identification of a high-risk cohort requiring transfer to tertiary centres that offer liver transplantation, rather than as definitive transplant listing criteria (see **chapter 4**).

Persistently elevated serum phosphate or reduced AFP levels may reflect failure of hepatic regeneration, and therefore could help predict a poorer outcome in paracetamol-induced ALF. Serum AFP showed high prognostic performance in one (Schmidt and Dalhoff 2005) study, whilst serum phosphate showed equivalence with the KCC in one (Bernal and Wendon 2003) retrospective study from KCH. Other authors, (in studies where transplantation was equated with death and hence excluded from this study), have demonstrated conflicting results with serum phosphate,(Gow, Sood, Angus 2003; Macquillan and others 2005; Ng, Davidson, Bathgate 2004; Schmidt and Dalhoff 2002) so further evaluation of both serum AFP and phosphate in future studies would be worthwhile. The MELD scoring system has been widely adopted for organ allocation in chronic liver disease and has shown encouraging prognostic accuracy in non-paracetamol ALF.(Dhiman and others 2007; Katoonizadeh and others 2007; Kremers and others 2004; Wiesner 2004) More limited data exist regarding the use of MELD in paracetamol-induced

ALF, but one additional study (excluded from this analysis as the 2x2 table was not reconstructable) suggested that its use may be limited by a high false positive rate.(Wei and others 2007)

In summary, this systematic review and meta-analysis has demonstrated that the original KCC for paracetamol-induced ALF have high pooled specificity, but low pooled sensitivity in determining prognosis in patients with paracetamol-induced ALF. The KCC had reduced prognostic accuracy when applied outside of KCH and were occasionally applied only at admission. The reduced specificity of the KCC following the addition of arterial lactate calls into question the benefit of this modification, suggesting that re-evaluation of this as a prognostic marker is required. Urgent consideration should be given to the design of a high-quality, prospective study evaluating the KCC, APACHE II scores, and markers of hepatic regeneration, such as serum AFP and phosphate, in paracetamol-induced ALF. Given the relatively rare nature of ALF, such a study is likely to require cooperation between several large centres, and argues the need for a collaborative network of tertiary hospitals experienced in the management and prognostication of ALF, similar to that developed by the Acute Respiratory Distress Syndrome Network program.(Anonymous 2000)

Chapter 3: Overdose pattern and outcome in paracetamol-induced acute severe hepatotoxicity

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Chapter 3: Overdose pattern and outcome in paracetamol-induced acute severe hepatotoxicity

3.1 Introduction

Previous studies and chapter 1 have highlighted the major contribution of paracetamol as a cause of ALF in the UK, North America, and Europe.(Bernal 2003; Larson and others 2005; Wei and others 2007) However, there are significant differences in the epidemiology of paracetamol-induced ALF in North America compared with the UK. Data from the USA, covering 1990-1999, suggested paracetamol overdose was responsible for 56,000 emergency department visits, 26,000 hospital admissions, and 458 deaths each year.(Nourjah and others 2006) Approximately 12,650 (22.6%) emergency department visits, 2240 (8.6%) admissions, and 100 (21.8%) deaths were due to unintentional paracetamol ingestion.(Nourjah and others 2006) Against this background the US Acute Liver Failure Study Group reported that unintentional overdose was the most common pattern of ingestion in patients with ALF, responsible for 48% of all overdoses, and reported similar outcomes between intentional and unintentional overdoses.(Larson and others 2005) These data contrast with the pattern of overdose reported in the UK. In the KCH series, 92% of paracetamol-induced ALF occurred after ingestion at a single time point with suicidal intent.(Makin, Wendon, Williams 1995) Contradictory data have also been presented regarding the outcome of ALF induced by accidental (unintentional) overdose of paracetamol, with increased mortality,(Gyamlani and Parikh 2002) or similar outcomes,(Larson and others 2005; Makin and Williams 2000) being reported. Lastly, in those series reporting increased mortality in patients following accidental paracetamol poisoning, it is unclear if this excess mortality is associated with this pattern of overdose *per se* or other clinical features such as organ failure or alcohol consumption prevalent in this population.(Gyamlani and Parikh 2002)

The aim of this cohort study was to analyse the incidence and outcome of unintentional paracetamol overdoses compared with intentional overdoses utilising prospectively defined data collected from 938 patients with acute severe liver injury admitted to the Scottish Liver Transplantation Unit (SLTU).

3.2 **Patients, methods, and definitions**

3.2.1 Patients

The cohort retrospectively analysed were from 938 patients admitted to the SLTU between 1st November 1992 and 31st October 2008 with suspected severe acute liver injury. Severe acute liver injury was defined as sudden deterioration in liver function with associated coagulopathy in the absence of a history of chronic liver disease, whilst the term ALF (i.e. fulminant liver failure) was restricted to those patients developing HE.(O'Grady, Schalm, Williams 1993) Guidelines for accepting patients from referring hospitals were based on previously published criteria and have remained unchanged over the time course of the study.(Bernal and others 1998) These admission criteria included: HE; progressive coagulopathy with a PT >50 seconds; INR >5; or in the case of paracetamol overdose, PT in seconds greater than time in hours post overdose; persistent metabolic acidosis despite adequate fluid resuscitation; hypoglycaemia; or deteriorating renal function in the presence of severe liver injury. Following admission a detailed clinical history, examination, and laboratory investigations were performed, with imaging studies and transjugular liver biopsy undertaken where clinically indicated. Laboratory investigations were repeated at daily intervals or more frequently in patients with rapidly progressive liver failure.

Patients admitted to the SLTU are managed using a standard protocol as previously described, which is reviewed on an annual basis.(Simpson and others 2009) Patients with paracetamol poisoning have continuous infusion of NAC (6.25mg/kg/hr) until the INR is less than 2. Coagulopathy is not routinely corrected unless there is excessive bleeding. Hypotension is treated with volume expansion and inotropic support and monitored by frequent hemodynamic assessment. Elective ventilation is initiated in patients with Grade III or IV HE or where there is progressive respiratory failure. Renal replacement therapy (RRT) is instituted utilising continuous veno-venous hemofiltration in oliguric patients with progressive rise in serum creatinine or those who are anuric. Antibiotic and anti-fungal treatments are prescribed in all patients admitted to the intensive care unit. Nutrition is provided by nasogastric feed if the patient was intubated. Intra-cranial pressure monitoring is considered in all intubated and ventilated patients who may be transplant candidates. Treatments for cerebral oedema include mannitol, therapeutic hypothermia, and thiopentone.

The KCC are used in this unit and throughout the UK to determine patients who will most likely die without OLT.(O'Grady and others 1989) The KCC were modified in 2006 within the UK to include arterial lactate concentration in patients with paracetamol overdose, as reviewed in chapter 2.(Bernal and others 2002) OLT was considered in all patients meeting the modified KCC in conjunction with their medical condition and psychological assessment. If accepted as transplant candidates, patients are 'super-urgently' listed with UK Transplant and prioritised for the next available compatible organ.

3.2.2 Methods

Since its inception in 1992, the SLTU has prospectively collected and maintained a dedicated database of all acute liver injury patients admitted to the unit. Data recorded in this database includes details of patient history, clinical examination, and laboratory results along with therapeutic interventions, including intensive care admission, need for RRT, or inotropic support. The following variables were recorded at the time of SLTU admission: temperature, pulse, white cell count (WCC), platelet count, INR, serum electrolytes, serum bilirubin, serum ALT, serum albumin, arterial hydrogen ion, bicarbonate, and arterial lactate. Where available, the paracetamol preparation, admission hospital serum paracetamol level, number of tablets, type (whether accidental or intentional) and timing of overdose, delay to presentation, and use of NAC were all recorded. Background information such as alcohol use and dependency, illicit drug use, pre-existing psychiatric history, and employment was obtained by the admitting medical team from a variety of sources including, where possible, the patient, the patient's family, and the patient's general medical practitioner. The suicidal ideation of each patient was assessed by detailed interview of the patient (when the absence of HE permitted this) by the specialist transplant psychiatric liaison team prior to any decisions regarding listing for OLT, with corroborating evidence obtained from the patient's family and general practitioner where possible. Where available, further information was obtained from review of the medical and psychiatric notes from the referring hospital.

3.2.3 Definitions

Paracetamol overdose was prospectively assigned as the cause of acute severe liver injury if there was a clear history of ingestion of potentially toxic amounts of paracetamol (>4g/day) within 7 days of presentation; admission hospital serum paracetamol levels were >10mg/L; or serum ALT level was >1000 IU/L within 7 days of a history of paracetamol ingestion

irrespective of the serum paracetamol concentration.(Larson and others 2005) Paracetamol overdose was only accepted as the cause of acute severe liver injury after exclusion of other potential aetiologies, in particular the presence of other hepatotoxic drugs or substances, hepatitis A and B, autoimmune hepatitis, and Wilson's disease.

An *intentional overdose* was defined as a cumulative dose of paracetamol >4 grams ingested over 4 hours or less with the objective of self-harm; *unintentional overdose* was defined as a paracetamol overdose ingested when self-harm was not the aim. *Single overdose* was an overdose (>4g) taken at a single defined time point whilst a *staggered overdose* described ingestion of two or more supratherapeutic paracetamol doses over a time interval of greater than eight hours resulting in a cumulative dose of >4 grams per day. *Mixed overdose* described more than one type of tablet being taken at or during the time of the overdose, whilst *compound overdose* described overdose of compound tablets which included paracetamol such as co-proxamol or co-dydramol. *Alcohol abuse* was locally defined as alcohol consumption >56 units/week for men and >42 units/week for women, twice the current Governmental sensible drinking limits.(Department of Health 1995) Outcome was defined as spontaneous survival without transplant, death without transplant, survival with transplant, and death with transplant. When undertaking survival analysis, survival was assessed at 30 days or at hospital discharge if this occurred earlier, whilst death and OLT were considered equivalent.

3.3 Statistical analysis

All patient data were prospectively recorded in the SLTU ALF database. Statistical analysis was retrospectively performed using SPSS software (SPSS 16.0, Chicago IL, USA) and Graphpad Prism (GraphPad Software Inc., La Jolla, CA). Data values are presented as median and interquartile range (IQR) or percentages unless otherwise stated. Continuous data were compared using analysis of variance or the Kruskal-Wallis test for non-normally distributed variables. Categorical data were analysed using Chi-squared tests or Fishers exact test. Stepwise logistic regression was used to determine factors predictive of death or LT in paracetamol-induced acute severe liver injury patients. Only variables with p <0.10 were included in the multivariate analysis. Actuarial probability curves were constructed

using the Kaplan-Meier method and compared with log-rank testing. A two-sided p value of less than 0.05 was considered statistically significant.

3.4 Results

3.4.1 Overall study population

Over a 16 year period (November 12th, 1992 -November 11th, 2008) 938 patients were admitted to the SLTU with suspected severe acute liver injury, of whom 663 (70.7%) were prospectively classified as having paracetamol-induced hepatotoxicity. 614 (92.6%) had a history of potentially toxic paracetamol consumption (>4g/day). 628 patients (94.7%) had an ALT >1000 IU/L (ALT >3500 in 526 (79.3%)) and 512 (77.2%) had detectable paracetamol in serum. Only 4 of these 663 patients (0.6%) fulfilled only one criterion for paracetamol-induced liver injury. All 4 of these patients had a history of ingestion of potentially toxic quantities of paracetamol but serum paracetamol was undetectable and ALT<1000 IU/L. Two of these patients had a clear history of a single ingestion of a large quantity of paracetamol with suicidal intent and two had a history of staggered unintentional paracetamol ingestion. The latter two became encephalopathic, one fulfilled KCC and both died without transplant. The two former patients survived without OLT. These 4 patients have been included in subsequent analysis as paracetamol cases. Baseline demographic and clinical characteristics of the paracetamol study group are outlined in **table 3.1**.

SLTU admission characteristic (n= 663 unless otherwise stated)	Value
Sex (male/female)	315/348 (47.5/52.5%)
Age (years)	34 (26-44)
Pattern of overdose Intentional Accidental Unknown	 500 (75.4%) 110 (16.6%) 53 (8.0%)
Time course of overdose Single Staggered Unknown	 450 (67.9%) 161 (24.3%) 52 (7.8%)
Paracetamol level (mg/L) (n=561)	60.5 (20-130)
Mixed overdose (n=620)	316 (51.0%)
Associated alcohol with overdose (n=590)	264 (44.7%)
Alcohol abuse† (n=581)	263 (45.3%)
Previous psychiatric history (n=577)	244 (42.3%)
Active drug abuse (n=623)	96 (15.4%)
Previous overdose (n=619)	242 (39.1%)
Unemployed at time of overdose (n=606)	289 (47.7%)
Time from overdose to SLTU admission (hours) (n=414)	52 (40-67)

Table 3.1. (continued overleaf) Admission characteristics of 663 subjects with paracetamol-induced acute severe liver injury.

Data are presented as median (IQR) or numbers (%) as appropriate.
† >56 units/week (male); >42 units/week (female)
Abbreviations: WCC, white cell count; ALT, alanine aminotransferase; PT, prothrombin time; SLTU, Scottish Liver Transplantation Unit.

SLTU admission characteristic (n= 663 unless otherwise stated)		Value
Received NAC in referring hospital (n=644)		559 (86.8%)
Admission laboratory parameters	WCC (x10⁹/L)	10.7 (7.6-14.5)
	Platelets (x10⁹/L)	125 (73-174)
	Creatinine (umol/L)	134 (84-234)
	ALT (IU/L)	7291 (4250-10130)
	Bilirubin (umol/L)	83 (58-113)
	Albumin (g/L)	35 (31-39)
	PT (seconds)	48 (34-67)
Ever encephalopathic Never encephalopathic Not encephalopathic on admission Developed encephalopathy during admission (n=362)		344 (51.9%) 319 (48.1%) 362 (54.6%) 43 (11.9%)
Overall outcome: Survived without transplant Died without transplant Survived with transplantation (to hospital discharge) Died with transplantation		 446 (67.3%) 165 (24.9%) 37 (5.6%) 15 (2.2%)

Table 3.1 (continued). Admission characteristics of 663 subjects with paracetamol-induced acute severe liver injury.

Data are presented as median (IQR) or numbers (%) as appropriate.
† >56 units/week (male); >42 units/week (female)
Abbreviations: WCC, white cell count; ALT, alanine aminotransferase; PT, prothrombin time; SLTU, Scottish Liver Transplantation Unit.

3.4.2 Clinical presentation

Of the 663 paracetamol cases, 520 (78.4%) had been transferred to the SLTU from a total of 14 separate health authorities, with the remaining 143 patients transferred from local hospitals or from wards within the Royal Infirmary of Edinburgh. In those patients (414/663, 62.4%) in whom accurate timings could be obtained, presentation to emergency services occurred at a median of 23 hours post final paracetamol ingestion (range 1-130 hours). Female admissions accounted for 348 (52.5%) of admissions, with 315 (47.5%) males admitted. The median age at admission was 35 (IQR 27-45) years for males and 34 (IQR 24-44) years for females. Information regarding NAC use in the referring hospital was available for 644/663 (97.1%) of patients, of whom 559 (86.8%) had received intravenous NAC, at a median time from last paracetamol ingestion of 23.75 (IQR 10-44) hours. A total of 263/581 (45.3%) patients had a history of chronic alcohol abuse, with 264/590 (44.7%) of patients taking alcohol concomitantly with their overdose. In those patients (n=606) in whom an employment history could be obtained, 289 (47.7%) patients were unemployed, 220 (36.3%) were employed, and 27 (4.5%) were in full time education. A total of 244/577 (42.3%) patients had a prior history of psychiatric illness and 242/619 (39.1%) had taken a previous overdose. A total of 96/623 (15.4%) patients admitted to current recreational drug use. A total of 301 (45.4%) of paracetamol-induced acute liver injury patients were encephalopathic on admission, and a further 43/362 (11.9%) went on to develop HE during admission. A total of 344 (51.9%) of patients therefore developed HE, and thus ALF, at some point during their illness.

3.4.3 Intentional versus unintentional overdoses

Of the 610 (92%) patients in whom a clear overdose history could be obtained, 500/610 (82%) reported an intentional (suicidal) overdose, whilst 110/610 (18%) subjects denied suicidal ideation and were classified as unintentional overdoses (**table 3.2**). Unintentional overdose subjects were significantly older (median 40 (IQR 30-48) years) compared with intentional overdose patients (33 (24-43) years, $p<0.001$), had a lower admission paracetamol level (34.7 (15.9-57.5) mg/L vs. 75.6 (25.4-148.2) mg/L. $p<0.001$), and were more likely to have consumed narcotic/paracetamol compound analgesics (37.6% vs. 24.7%, $p<0.001$). Information regarding the reasons for overdose was available for 82/110 (74.5%) of unintentional overdoses. The most common rationale for overdose was for relief of pain, including abdominal pain (n=26), headache (n=17), musculoskeletal pain (n=17), toothache (n=5), chest

pain (n=2), and dysmenorrhoea (n=1). Other causes for overdose included accidental overdose during chemical intoxication (n=5), non-specific systemic illness (n=4), limb abscess (n=2), iatrogenic overdose (n=2), and one overdose taken unintentionally by a patient with cognitive impairment. Of the 52 subjects who had consumed compound analgesics or taken mixed overdoses unintentionally, the majority (29/52, 55.8%) had used codeine phosphate/paracetamol compounds (co-codamol), an analgesic available over the counter (OTC) in pharmacies in the UK at a dose of 8/500mg, and only by prescription at higher doses. A total of 8 subjects had overdosed on prescribed compound analgesics, namely dextropropoxyphene/paracetamol (coproxamol, n=5) and dihydrocodeine tartrate/paracetamol (codydramol, n=3). A total of 5 cases had overdosed on both co-codamol and coproxamol. The remaining compound overdose cases had used aspirin/paracetamol OTC compounds (n=6). Of the mixed overdoses, 3 cases had taken non-steroidal anti-inflammatories and paracetamol, 2 had used aspirin and paracetamol, whilst a further 3 cases had taken paracetamol with benzodiazepines. The remaining 6 cases had taken mixed overdoses of other prescription medications and paracetamol.

Unintentional overdose patients were significantly more likely to have consumed paracetamol in a staggered fashion (90.8% vs. 10.6%, $p<0.001$), and to have taken a lower cumulative paracetamol dose (11 (5-29) g vs. 27.5 (20-45) g, $p<0.001$). Unintentional overdose subjects were also more likely to have a history of chronic alcohol abuse (40.8% vs. 20.8%, $p<0.001$), and to have consumed alcohol with their overdose (52.5% vs. 42.7%, $p=0.037$), but were less likely to have a prior psychiatric history (24.5% vs. 46.2%, $p<0.001$). Unintentional overdose subjects had significantly lower admission ALT levels (3931 (2036-7184) IU/L vs. 8295 (5257-10920) IU/L, $p<0.001$) but had significantly more deranged serum sodium (133 (130-137) mmol/L vs. 136 (133-138), $p=0.001$), creatinine (203 (110-327) μmol/L vs. 114 (81-207) μmol/L, $p<0.001$), and albumin (31 (24-35) g/L vs. 37 (33-41) g/L, $p<0.001$) levels. Subjects consuming paracetamol unintentionally were significantly less likely to have received treatment with NAC prior to transfer to the SLTU (80.4% vs. 89.6%, $p=0.008$). Unintentional overdose patients were more likely to develop HE (59.1% vs. 47.4%, $p=0.027$) and had more systemic organ failure, such as requirement for renal replacement therapy (41.8% vs. 28.4%, $p=0.048$), or need for mechanical ventilation (52.7% vs. 38%, $p=0.005$), than intentional overdose patients. Unintentional overdose subjects were also more likely to fulfil the KCC (36.4% vs. 26%, $p=0.034$), but were subsequently less likely to undergo OLT (15% vs. 32.3%,

p=0.044). Overall spontaneous survival (57.3% vs. 74.4%, p<0.0001) was significantly worse in the unintentional patient group (**figure 3.1**). Similar results were obtained when transplanted patients were excluded from analysis (X^2=18.0, p<0.0001, **figure 3.2**). Subgroup analysis of ALF patients (n=344) revealed significantly lower spontaneous survival rates amongst unintentional (30.7%) and unknown (16.7%) overdose cohorts compared with intentional overdoses (45.6%, X^2=14.3, p=0.008, **figure 3.3).**

Variable	Intentional	N	Unintentional	N	*P* value
Sex (male/female)	248/252 (49.6/50.4%)	500	50/60 (45.5/54.5%)	110	0.431
Age (years)	33 (24-43)		40 (30-48)		<0.001
Paracetamol level (mg/L)	75.6 (25.4-148.2)	432	34.7 (15.9-57.5)	88	<0.001
Paracetamol dose ingested (g) (range)	27.5 (20-45) Range (4-150)	500	11 (5-29) Range (4-70)	97	<0.001
Paracetamol only **Compound narcotic/paracetamol use** **Mixed overdose**	237 (48.1%) 122 (24.7%) 134 (27.2%)	493	43 (45.3%) 38 (40%) 14 (14.7%)	95	<0.001
Associated alcohol ‡	196 (42.7%)	459	52 (52.5%)	99	0.037
Alcohol abuse†	101 (20.8%)	485	42 (40.8%)	103	<0.001
Staggered overdose	53 (10.6%)	499	99 (90.8%)	109	<0.001
Previous psychiatric history	206 (46.2%)	446	23 (24.5%)	94	<0.001
Active drug use	80 (16.7%)	478	(10.5%)	105	0.110
Received NAC in referring hospital	441 (89.6%)	492	86 (80.4%)	107	0.008

Table 3.2. (continued overleaf) Admission clinical and laboratory data in patients with intentional or unintentional paracetamol overdose.

Variable		Intentional (N=500)	Unintentional (N=110)	*P* value
Admission laboratory parameters	Platelets (x10⁹/L)	129 (81-176)	113 (62-169)	0.043
	Sodium (mmol/L)	136 (133-138)	133 (130-137)	<0.001
	Creatinine (μmol/L)	114 (81-207)	203 (110-327)	<0.001
	ALT (IU/L)	8295 (5257-10920)	3931 (2036-7184)	<0.001
	Bilirubin (μmol/L)	84 (58-112)	75 (58-118)	0.530
	Albumin (g/L)	37 (33-41)	31 (24-35)	<0.001
	PT (seconds)	48 (34-67)	47 (33-64)	0.540
Developed encephalopathy		237 (47.4%)	65 (59.1%)	0.027
Met King's College Criteria		130 (26%)	40 (36.4%)	0.034
CVVH		141 (28.4%)	46 (41.8%)	0.048
Mechanical ventilation		190 (38%)	58 (52.7%)	0.005
Transplanted		42 (8.4%)	6 (5.5%)	0.044
Spontaneously survived		372 (74.4%)	63 (57.3%)	<0.001

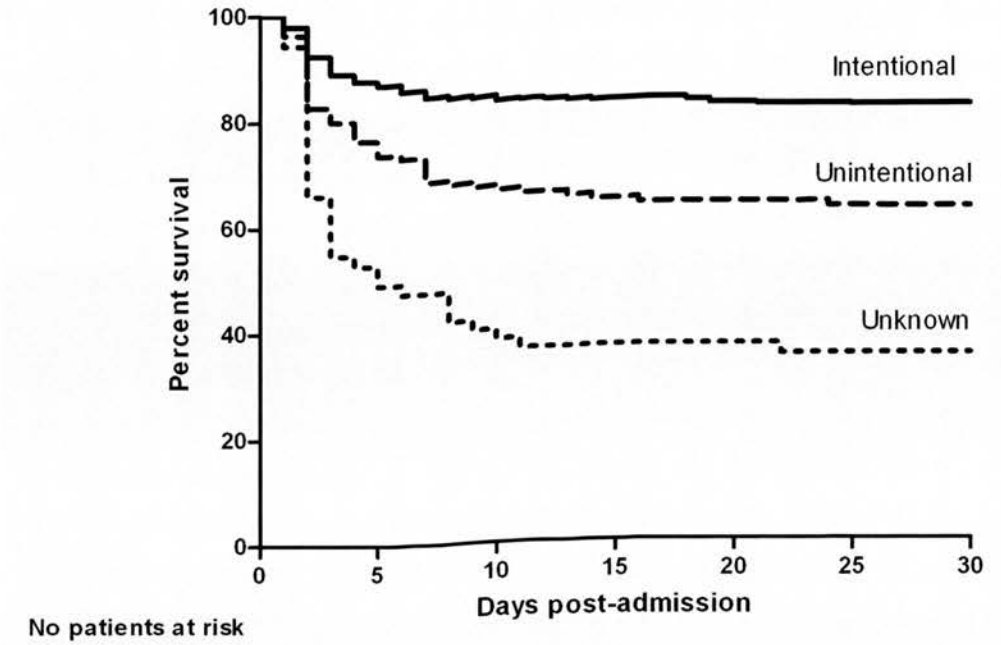
Table 3.2. continued. Admission clinical and laboratory data in patients with intentional or unintentional paracetamol overdose.

Data are on admission to the SLTU unless otherwise stated and are presented as median (IQR) or numbers (%) as appropriate.

† >56 units/week (male); >42 units/week (female) ‡ Alcohol taken with paracetamol overdose.

3.4.4 Patients with unobtainable overdose history

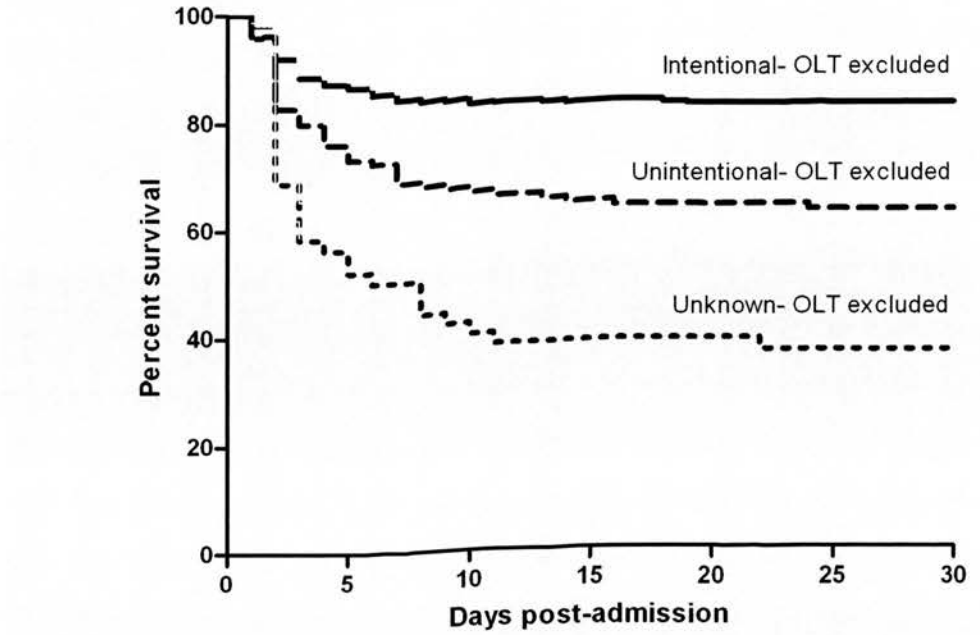
In 53 (8.0%) patients, no clear history of suicidal intention (or otherwise) could be obtained, despite attempts to interview the patient, the patient's family, and by liaison with the referring hospital. In the majority (72%) of cases, this was due to the patient having HE on arrival and being unable to provide a history. These patients tended to be older (median 40 (33-50) years vs. 34 (25-44) years, p=0.020) and were more likely to be female (67.9% vs. 51.1%, p=0.033) compared with the other 610 overdose patients. However, there were no significant differences between these two groups in: the number of tablets consumed; the proportions consuming alcohol concomitantly with their overdose; the proportions with a previous overdose history; the proportions with a history of drug abuse. Of the 53 patients in whom a history was unobtainable, 19 (35.8%) were mechanically ventilated on arrival, 7 (13.2%) were in grade III-IV HE, and 12 (22.6%) had grade I-II HE. These 53 patients required significant levels of organ support, with 39 (73.6%) requiring mechanical ventilation, 29 (54.7%) renal replacement therapy, and 30 (56.6%) pressor support. These patients had a particularly poor clinical outcome, with 27 (50.9%) fulfilling the KCC, 31 (58.5%) dying without transplantation, and only 17 (32.1%) spontaneously surviving (**figure 3.1**).



No patients at risk							
Intentional	500	445	416	405	401	397	396
Unintentional	110	84	73	70	69	68	67
Unknown	53	28	22	20	19	19	18

Figure 3.1. Survival curves of patients with paracetamol-induced acute severe liver injury according to the pattern of overdose.

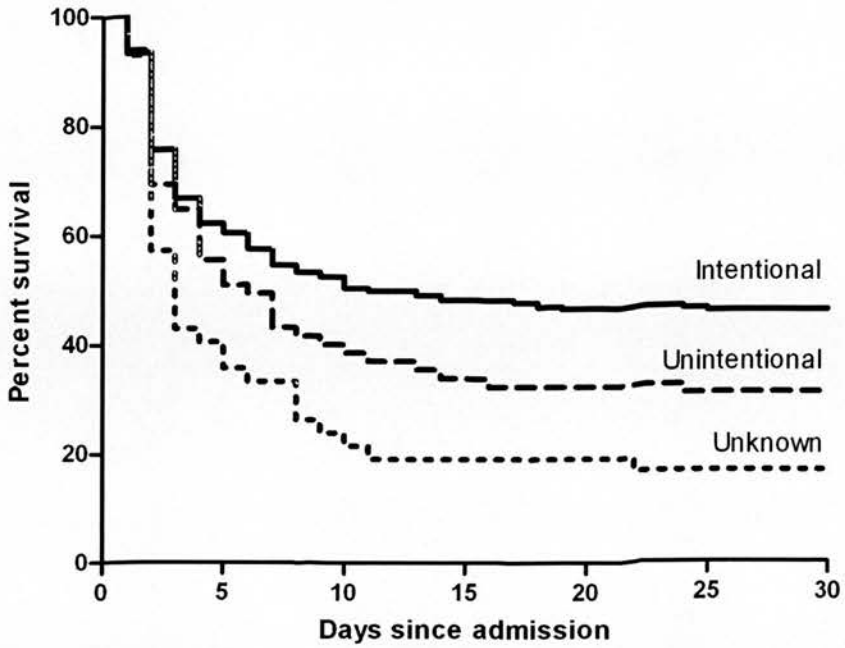
Survival curves were significantly different when compared using log-rank testing (p<0.0001). OLT was considered equivalent to death.



Intentional	458	399	378	370	366	366	365
Unintentional	104	79	69	66	65	64	63
Unknown	48	27	20	19	19	18	17

Figure 3.2. Survival curves of patients with paracetamol-induced acute severe liver injury according to overdose pattern, after exclusion of transplanted patients.

Survival curves were significantly different when compared using log-rank testing ($p<0.0001$).



Intentional	237	147	124	116	111	109	108
Unintentional	65	36	26	23	22	21	20
Unknown	42	17	10	9	9	8	7

Figure 3.3. Survival curves of patients with paracetamol-induced acute liver failure according to the pattern of overdose.

Survival curves were significantly different when compared using log-rank testing (p=0.0008). OLT was considered equivalent to death.

3.5 Prognostic accuracy of the KCC in different patterns of paracetamol overdose

The prognostic accuracy of the KCC was determined for the entire paracetamol ALF cohort (n=344), then separately for ALF patients with intentional (n=237) and unintentional (n=65) overdoses (**Table 3.3**). Although the KCC had high specificity for both overdose patterns, the sensitivity in predicting outcome was considerably better for intentional overdoses (89.9%, 95% confidence intervals (CI) 83.4-94.5) compared with unintentional overdoses (77.8%, 95% CI 62.9-88.8).

	KCC+ve/ deaths	Total deaths	Sensitivity (95% CI)	Specificity (95% CI)	LR+ (95% CI)	LR- (95% CI)	DOR (95% CI)
Paracetamol-ALF (n=344)	197/180	213	84.5 (81.3-87.0)	87.0 (81.8-91.1)	6.5 (4.5-9.8)	0.18 (0.14-0.23)	36.6 (19.5-68.4)
Intentional (n=237)	126/116	129	89.9 (83.4-94.5)	90.7 (83.6-95.5)	9.7 (5.4-17.6)	0.11 (0.07-0.19)	87.4 (36.7-208.1)
Unintentional (n=65)	38/35	45	77.8 (62.9-88.8)	85.0 (62.1-96.8)	5.2 (1.8-14.9)	0.26 (0.15-0.47)	19.8 (4.8-81.6)

Table 3.3 Prognostic accuracy of the KCC in paracetamol-induced ALF.

Transplanted patients have been included as having died.

+LR/-LR: +ve/-ve likelihood ratio; DOR: diagnostic odds ratio

3.6 Predictors of death in paracetamol-induced acute liver injury

In view of the significant differences in a number of prognostic variables between the different overdose subgroups, logistic regression analysis of SLTU admission parameters including patterns of overdose was performed to determine independent predictors of death or LT in paracetamol-induced acute severe liver injury (**Table 3.4**). Univariate analysis identified increasing age ($p<0.001$), WCC ($p<0.001$), PT ($p<0.001$), serum creatinine ($p<0.001$), H^+ ($p<0.001$), and hyponatraemia ($p=0.011$) as potential predictors of death/OLT. Unintentional overdoses ($p=0.001$), the absence of clinical history ($p<0.001$), the presence of encephalopathy on admission ($p<0.001$), and thrombocytopaenia ($p<0.001$) were also potentially significant after univariate analysis. Acute or chronic alcohol abuse was not predictive of a poorer outcome, nor was lack of treatment with NAC in the referring hospital. Multivariate analysis identified encephalopathy on admission (odds ratio (OR) 4.50, 95% confidence intervals (CI) 2.76-7.34), increasing WCC (OR 1.04; 95% CI 1.02-1.06), admission PT (OR 1.03; 95% CI 1.02-1.04), and admission creatinine (OR 1.00; 95% CI 1.00-1.01) as independently predictive of death/OLT. Both unintentional overdoses (OR 1.91; 95% CI 1.07-3.43), and overdoses where there was no reliable history (OR 6.65; 95% CI 1.78-24.81) were independently predictive of a poor outcome. Other independent predictors were increasing age (OR 1.04; 95% CI 1.02-1.06), and thrombocytopaenia (OR 0.99; 95% CI 0.99-1.00).

Variable	Univariate OR (95% CI)	*p* Value	Multivariate OR (95% CI)	*p* Value
Unintentional overdose	1.29 (1.09-1.53)	0.001	1.91 (1.07-3.43)	0.032
Overdose history unavailable	2.52 (1.98-3.21)	<0.001	6.65 (1.78-24.81)	0.005
Age	1.04 (1.02-1.05)	<0.001	1.04 (1.02- 1.06)	<0.001
Concomitant alcohol with OD	1.00 (1.00- 1.00)	0.527	NA	
Chronic alcohol abuse	1.34 (0.79-2.26)	0.274	NA	
Not given NAC in referring hospital	1.37 (0.84-2.24)	0.210	NA	
Encephalopathy on admission	5.50 (3.55-8.53)	<0.001	4.50 (2.76-7.34)	<0.001
Admission WCC	1.11 (1.08-1.15)	<0.001	1.04 (1.02- 1.06)	<0.001
Admission platelet count	0.99 (0.99- 1.00)	<0.001	0.99 (0.99- 1.00)	0.012
Admission PT	1.03 (1.02-1.03)	<0.001	1.03 (1.02- 1.04)	<0.001
Admission sodium	0.96 (0.92-0.99)	0.011	0.99 (0.94-1.03)	0.572
Admission creatinine	1.01 (1.01-1.01)	<0.001	1.00 (1.00- 1.01)	<0.001
Admission H^+	1.08 (1.06- 1.10)	<0.001	1.08 (0.97- 1.21)	0.180

Table 3.4: Factors predictive of mortality on univariate and multivariate analysis of admission parameters in patients with paracetamol-induced acute severe liver injury.

OLT was considered equivalent to death.

3.7 **Discussion**

In this chapter the impact of suicidal ideation upon patient outcome is analysed using a large cohort of paracetamol-induced acute severe liver injury patients. Using prospective definitions of overdose pattern, intentional (suicidal) overdose was the commonest pattern of paracetamol ingestion, accounting for 75.4% of all paracetamol cases. However, despite lower admission paracetamol and ALT levels, patients with unintentional overdose had significantly reduced survival compared with intentional overdoses. Additionally, the KCC were less sensitive in predicting outcome in unintentional overdose cases. Both unintentional overdoses and 'unknown' overdoses, where no clear history could be obtained, were independently associated with increased mortality. These data suggest that the pattern of overdose should be taken into account when assessing patients with paracetamol-induced hepatotoxicity, and that, irrespective of their admission paracetamol levels, those patients with unintentional overdose should be managed as high-risk cases due to their significantly increased mortality.

The strengths of this study include the large number of patients, the single centre nature of the study, and the prospectively defined criteria of overdose. The SLTU represents a single referral and management facility for all patients in Scotland with ALF irrespective of their suitability for liver transplantation, and the Scottish population has remained relatively stable at 5.1 million over the period of the study. However, not all ALF cases occurring in Scotland will have been transferred to the SLTU during the course of the study due to medical instability precluding safe patient transfer.(Blair and others 1998) Criteria for patient admission have remained largely unchanged during the time course of the study, further reducing patient heterogeneity, a recognized problem in previous cohort studies of paracetamol hepatotoxicity.(Schiodt and others 1997; Walker 1998) The overall mortality rate of 32.7% represents selection bias for the more severe paracetamol cases in Scotland, since admissions to the SLTU are determined by severity of liver dysfunction, rather than on the basis of a history of paracetamol consumption or number of tablets consumed. This latter point is of particular note since intentional and unintentional paracetamol overdoses represent considerably different patient populations with regards to demographics, timing of presentation, and degree of organ dysfunction at presentation. Suicidal patients often present to a hospital setting as a direct result of the psychological consequences of their

overdose, rather than as a result of symptoms; in contrast, unintentional overdoses usually present because of morbidity, and therefore tend to be systemically unwell at presentation. Our study partially eliminates this problem through a gatekeeper mechanism (see **chapter 4**), but inevitably introduces referral bias, since only those patients with potentially reversible organ failure are accepted.

Defining paracetamol as a cause of hepatotoxicity is recognised as particularly difficult in cases where there is no obvious suicidal intent or patients are unable to give a history. Prospective definitions were used for paracetamol hepatotoxicity and overdose subgroups, but some of the unintentional overdoses in our cohort may still represent other, unidentified, primary causes of hepatotoxicity in whom paracetamol was taken to relieve systemic malaise. However, paracetamol overdose was not simply used as a 'catch-all' diagnosis in the absence of an alternative diagnosis- seronegative hepatitis represented 63/938 (6.7%) of all cases during this study, none of whom were classified as paracetamol-induced hepatotoxicity (data not shown). It is also recognised that some unintentional cases will have been disguised suicides, especially since 'accidental' overdoses are associated with both chronic alcohol abuse and underlying depression.(Makin and Williams 2000) Indeed, this analysis would suggest potential suicidal motives behind large overdoses of paracetamol, particularly if not taken as a compound analgesic. The ability and time available to obtain a detailed psychiatric history to confirm intent is limited by the rapid clinical progression of paracetamol-induced ALF, and by the development of HE, but, given that the lack of a detailed history regarding suicidal intent is in itself an independent predictor of poor prognosis, this further emphasises that such cases should be treated as high-risk.

Previous studies have suggested that accidental paracetamol overdose is associated with increased mortality.(Gyamlani and Parikh 2002; Schiodt and others 1997) However, this poorer prognosis is not a universal finding in other cohort studies of paracetamol hepatotoxicity.(Larson and others 2005; Makin and Williams 2000) The recently published multicentre cohort analysis from the US Acute Liver Failure Study group found similar survival in 131 unintentional patients (72% 3 week survival) compared with 122 intentional patients (71% 3 week survival).(Larson and others 2005) This difference in survival compared with these data does not relate to differences in transplant rates, but may be due to the increased frequency of renal dysfunction in the SLTU cohort compared with the US

series. It was recently reported that renal dysfunction on admission in patients with ALF is a significant predictor of poor outcome in this cohort.(Leithead and others 2009) Another important potential confounding factor is alcohol abuse, given that unintentional overdose patients in our cohort were not only more likely to abuse alcohol chronically, but were also more likely to consume alcohol acutely at the time of overdose. However, neither acute nor chronic alcohol abuse were independent predictors of death or OLT on multivariate analysis. The association of alcoholism with other confounding factors, such as delayed presentation, increased paracetamol dosage, and older age, may account for the poorer outcomes previously reported in alcoholics following paracetamol overdose. (Schiodt and others 2002; Schmidt, Dalhoff, Poulsen 2002) Furthermore, since alcoholism is a recognised risk factor for disguised suicidal overdose,(Suokas and Lonnqvist 1995) some parasuicidal attempts in alcoholic patients may have been misclassified as unintentional overdoses. Another important element in the prognosis of unintentional overdoses is likely to be the time period prior to treatment with NAC ('time to NAC'). These data demonstrate that unintentional overdoses are less likely to receive NAC in the referring hospital, although this was not an independent risk factor for poor outcome. Delayed 'time to NAC' is a recognised independent risk factor for poor outcome following single time point paracetamol overdose,(Schmidt, Dalhoff, Poulsen 2002) and delayed presentation is thought to be more common amongst alcoholics.(Read, Tredger, Williams 1986) Given the increased frequency of both alcoholism and staggered overdoses amongst the unintentional overdose cohort, it is likely that absence of, or delayed treatment with, NAC in the referring hospital was at least partially responsible for the poorer outcome in this group. As a result, patients with acute liver injury and a history of unintentional supratherapeutic paracetamol ingestion should be treated as high-risk irrespective of serum paracetamol levels, and commenced on NAC treatment whilst other aetiological investigations are pending.

Admission HE, coagulopathy, renal failure, thrombocytopaenia, leucocytosis, and increasing age, as well as unintentional or unknown patterns of overdose, predicted a poorer outcome in the paracetamol overdose patients as a whole. Whilst many of these factors are well established as predicting a poorer outcome in paracetamol-induced ALF,(O'Grady and others 1989) the impact of overdose pattern upon outcome has not been examined in depth. Reanalysis of the data including only ALF patients, or after exclusion of transplanted patients, did not significantly affect the impact of overdose pattern upon outcome. The

effects of overdose pattern appear to be independent of patient age, alcohol intake, renal failure, and HE, but this requires confirmation in other patient cohorts. The increasingly recognised deleterious effects of the systemic inflammatory response following paracetamol hepatotoxicity may explain the predictive nature of leucocytosis seen in this study.(Liu, Govindarajan, Kaplowitz 2004; Rolando and others 2000; Schmidt and Larsen 2006) Increasing age has previously been recognized as an independent risk factor for poor outcome following paracetamol overdose,(Schmidt 2005) and the older age of the unintentional and unknown overdose cohorts may represent either an increased frequency of this deleterious overdose pattern amongst older patients, or a lower threshold for the development of severe hepatotoxicity following paracetamol overdose in older subjects.

There is an increased frequency of unintentional overdose in this cohort compared with data previously published from King's College in which only 8% of paracetamol overdoses were due to accidental ingestion.(Makin, Wendon, Williams 1995) Due to the potential selection bias associated with this study, it cannot be concluded that the nationwide incidence of unintentional paracetamol overdoses is increasing, but this question deserves attention due to the poor outcome seen in this overdose subgroup. The increased proportions of unintentional overdoses seen in this study compared with the King's College study may also reflect temporal changes associated with legislation affecting the availability and packaging of paracetamol that followed the publication of the King's College series,(Hawton and others 2004) or differences due to socioeconomic factors and the increased rates of chronic alcohol abuse in the Scottish population compared with the rest of the UK.(Leon and McCambridge 2006) The KCC identify patients with increased risk of death following paracetamol poisoning and are the "transplant criteria" in use throughout the UK to determine patient prognosis.(Bernal and others 2002; O'Grady and others 1989) Within the unintentional cohort the KCC are specific, but lack sensitivity, for paracetamol-induced ALF, suggesting that alternative prognostic criteria may be required following this pattern of overdose.(Antoniades and others 2006; Chung, Sitrin, Te 2003; Dabos and others 2005; Schiodt and others 2005; Schmidt and Dalhoff 2005; Schmidt and Larsen 2007; Yantorno and others 2007)

In conclusion, unintentional paracetamol overdose is independently associated with increased mortality compared with intentional overdose. This pattern of overdose is

associated with older age, acute and chronic alcohol abuse, and a staggered pattern of overdose. Despite lower admission ALT and paracetamol levels, unintentional overdose patients have increased systemic dysfunction and poorer clinical outcomes compared with intentional overdoses. The KCC are less sensitive in predicting outcome in unintentional overdose and alternative prognostic criteria may be required for this subgroup.

Chapter 4: The temporal evolution of the Systemic Inflammatory Response Syndrome (SIRS) and Sequential Organ Failure Assessment (SOFA) scores following paracetamol overdose

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4.2 Patients, methods, and definitions

4.2.1 Prospective validation cohort

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4.3.1 SIRS

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4.6 Discussion

Chapter 4: The temporal evolution of the Systemic Inflammatory Response Syndrome (SIRS) and Sequential Organ Failure Assessment (SOFA) scores following paracetamol overdose

4.1 Introduction

The preceding chapters have outlined the importance of paracetamol as the main aetiological factor responsible for ALF in the developed world.(Larson and others 2005) Paracetamol-induced ALF is strongly associated with the development of the SIRS and a subsequent cascade of complications including HE, coagulopathy, and multiple organ dysfunction syndrome.(Rolando and others 2000a) As outlined in **chapter 2**, several studies have examined the prognostic accuracy of multiorgan failure assessment scores such as SOFA and APACHE II scores to predict outcome following paracetamol hepatotoxicity,(Cholongitas and others 2006a; Mitchell and others 1998; Schmidt and Larsen 2006) but these studies have tended to apply the prognostic test in question at the point of hospital admission, rather than in relation to the time from overdose. This inevitably introduces confounding since many patients present to hospital following paracetamol overdose due to psychological, rather than physical, morbidity. Therefore, a better understanding of the temporal evolution of the SIRS and multiorgan failure following paracetamol overdose could improve prognostication in this condition. No studies exist which examine the temporal relationship between the initial hepatotoxic insult (i.e. paracetamol overdose) and development of the SIRS and multiorgan failure. The aims of this chapter were to examine these temporal changes in a cohort of patients who required tertiary level care after taking a single intentional paracetamol overdose. It was hypothesised that the absence of a SIRS response or extrahepatic organ injury following overdose would be associated with a low risk of in hospital mortality, and that the SIRS and SOFA scores could therefore act as quantitative triage instruments to identify those patients at lower risk of requiring emergency OLT.

4.2 Patients, methods, and definitions

The cohort retrospectively analysed were 100 consecutive single time point intentional (defined as a paracetamol overdose (>4g) taken at a single defined time point with the

objective of self-harm) paracetamol overdoses admitted to the SLTU with acute liver injury between April 2003 and December 2009. Specifically exclusion criteria included patients with staggered overdoses, or overdoses taken accidentally in an attempt to relieve pain, due to the confounding temporal and diagnostic problems associated with these overdoses.(Gyamlani and Parikh 2002) Paracetamol overdose was defined as described in **chapter 3** as at least 2/3 of: a history of ingestion of potentially toxic amounts of paracetamol (>4g/day); detection of paracetamol in the serum >10mg/L; or a serum ALT level> 1000 IU/L within 7 days of a history of paracetamol ingestion irrespective of the serum paracetamol concentration.(Larson and others 2005) All 100 patients fulfilled all of these three criteria. Other definitions were as described in **chapter 3**. Since the purpose of this study was not to develop transplantation listing criteria, death and OLT were considered equivalent when undertaking survival analysis.

4.2.1 Prospective validation cohort

The prognostic accuracy and threshold values of the SIRS and SOFA scores identified by the retrospective analysis were prospectively validated in a cohort of paracetamol-induced acute liver injury patients admitted to the Royal Infirmary of Edinburgh between March 2009 and July 2010. Admission laboratory and clinical parameters, including temperature, encephalopathy grade, mean arterial pressure, leucocyte count, platelet count, INR, serum electrolytes, serum bilirubin, and serum ALT were prospectively recorded in a dedicated database, and the SOFA and SIRS scores calculated as described below. The clinical team caring for these patients was unaware of the results of this study.

4.3 Laboratory parameters

Retrospective analysis of all hospital electronic laboratory records was performed to obtain all standard haematological, biochemical, and coagulation samples obtained for each patient during the first 10 days of admission. The timing of each sample was noted from either the laboratory receipt or the clinical notes, and the corresponding haemodynamic and clinical parameters for each sampling time point retrieved from the clinical notes. These data were then individually compared against the reported time from overdose for each patient, and the laboratory and clinical values for each 6-hour period post-overdose recorded in a dedicated database. Where laboratory data were available more frequently than 6-hourly,

the most deranged laboratory values were used. The data from these 6-hour periods were then pooled to obtain median and IQRs for each parameter for patients who died or required OLT, and for patients who survived spontaneously.

4.3.1 SIRS

The presence of the SIRS was defined as two or more of: temperature <36°C or >38°C, heart rate >90 beats/minute, leucocyte count <4 x 10^9/L or >12x 10^9/L, and tachypnoea > 20 breaths/minute or $PaCO_2$ <4.3 kPa.(Anonymous 1992) The daily presence or absence of the SIRS, and the number of the SIRS components fulfilled, were retrospectively calculated for each patient by correlating the daily leucocyte count with the most abnormal temperature and heart rate recorded during each corresponding day of admission. Respiratory rate was only included for non-ventilated patients, as the majority of patients in the SLTU are electively mechanically ventilated following the development of grade III or IV HE. The presence of infection, defined as positive blood, urine, or sputum cultures and/or ascitic fluid polymorphonuclear count >250/mm^3 and/or radiological evidence of infection, was documented as were prescriptions for antimicrobial or antifungal agents.

4.3.2 Organ failure

Organ failure was assessed using the SOFA score, which assesses six organ systems; (hepatic, renal, coagulation, cardiovascular, respiratory, and central nervous) and provides a graded score from 0-4 points for each organ system (**Table 4.1**).(Vincent and others 1996) The SOFA score was calculated for each 24-hour period following overdose, with the most deranged values for each parameter in each 24 hour period used. Organ failure was defined as a SOFA score ≥3 points for the organ concerned (**Table 4.1**). In sedated patients, the assumed Glasgow Coma Score during periods of sedation hold was used to evaluate the neurological status. Due to potential confounding, SOFA scores were not calculated following the administration of platelet transfusions.

SOFA score					
Organ system	Score				
	0	1	2	3	4
Respiratory: PaO_2/FiO_2	>400	≤400	≤300	≤200	≤100
Renal: creatinine (μmol/l)	≤110	110–170	171–299	300–440; urine output ≤500 ml/day	>440; urine output <200 ml/day
Hepatic: bilirubin (μmol/l)	≤20	20–32	33–101	102–204	>204
Cardiovascular: hypotension	No hypotension	MAP <70 mmHg	Dopamine ≤5[a], dobutamine (any dose)	Dopamine >5[a] or epinephrine ≤0.1[a] or norepinephrine ≤0.1[a]	Dopamine >15[a] or epinephrine >0.1[a] or norepinephrine >0.1[a]
Hematologic: platelet count	>150	≤150	≤100	≤50	≤20
Neurologic: Glasgow Coma Scale score	15	13–14	10–12	6–9	<6

[a]Adrenergic agents administered for at least one hour (doses given are in μg/kg per minute). FiO_2, fractional inspired oxygen; MAP, mean arterial pressure; PaO_2, arterial oxygen tension; SOFA, Sequential Organ Failure Assessment.

Table 4.1 The Sequential Organ Failure Assessment (SOFA) score

4.4 Statistical Analysis

Statistical analysis was performed using SPSS (SPSS 16.0, Chicago IL, USA) and Graphpad Prism (GraphPad Software Inc., La Jolla, CA). Data values are presented as median +/- IQR or percentages unless otherwise stated. Outcome was defined as spontaneous survival to discharge without transplant, death without transplant, survival with transplant, and death with transplant. ROC analysis was used to identify optimum threshold values to discriminate non-survivors. Continuous data were compared using either analysis of variance or the Kruskal-Wallis test if inter-group variances were unequal, with post-hoc Dunn's testing used to compare selected groups. Categorical data were analysed using Chi-squared tests or Fishers exact test. Bonferroni corrections were undertaken for repeated measures. Results were considered statistically significant when $p < 0.05$.

4.5 **Results**

4.5.1 Patients and details of overdose

A total of 100 patients (46 male, 54 female) admitted to the SLTU between April 2003 and December 2009 were included in the study. During this period, a total of 347 patients had been admitted, of whom 111 were classified as 'non-paracetamol' cases, 62 patients had taken a staggered paracetamol overdose, 45 patients had overdosed on paracetamol accidently, and in the remaining 29 cases the details of the paracetamol overdose were unclear. The median patient age of the 100 included patients was 34 (IQR 24-43) years. A total of 83 patients were transferred to the SLTU from outlying health boards, at a median time of 50 (36-66.5) hours following overdose. The remaining patients were admitted to the SLTU from wards within the Royal Infirmary of Edinburgh or from local hospitals. The median ingested paracetamol dose was 25 (17.5-41) g. All 100 patients received NAC treatment at their local hospital, at a median time from overdose of 22.75 (9.5- 42.25) hours. A total of 47 (47%) of patients had taken a mixed overdose. As shown in **table 4.2**, none of these demographic parameters or potential confounders differed significantly between survivors and patients who died/required OLT. Other baseline demographic and clinical characteristics of the paracetamol study group are outlined in **table 4.2**.

Variable	Dead/OLT	n	Spontaneous survival	n	*p*
Sex (male/female)	6/15 (28.6%/71.4%)	21	40/39 (50.6%/49.4%)	79	0.071
Age (years)	37 (28-44)		33 (22-43)		0.115
Ingested paracetamol dose (g)	27.5 (15.5-40.0)		25.0 (17.0-40.5)		0.226
Admission paracetamol level (μmol/L)	56 (37-146)		70 (24-145)		0.545
Mixed OD	8 (38.1%)		39 (49.4%)		0.115
Previous OD	8 (57.1%)	14	29 (46.0%)	63	0.388
Active drug abuse	3 (14.3%)	21	11 (14.5%)	76	0.654
Weekly alcohol consumption (units)	50 (4-140)	13	20 (2-68)	57	0.182
Time from OD to NAC (hours)	15.5 (10.0-34.5)	21	23.5 (11.0-43.5)	79	0.791
Time from OD to SLTU admission (hours)	41.0 (29.5-49.0)		53.0 (45.5-67.0)		0.045

Table 4.2. Demographics of study population and details of paracetamol overdose.

Data are presented as median (+/- interquartile range) or percentages as appropriate

4.5.2 Hepatic encephalopathy & other outcomes

A total of 29 (29.0%) of paracetamol-induced acute liver injury patients were encephalopathic on admission to the SLTU, and a further 18/71 (25.4%) went on to develop HE during admission. A total of 47 (47%) patients therefore developed HE, and thus ALF, at some point during their illness. Of these patients, 22 (46.8%) subsequently met the modified KCC, at a median time from overdose of 51.5 (39-73) hours from overdose. Of these 22 patients, 3 were transplanted, 14 died without OLT, and 5 recovered spontaneously. A total of 4 patients died without meeting the modified KCC. The sensitivity, specificity, and DOR of the KCC were 81.0 (95% CI 65.0- 90.7), 93.7 (95% CI 89.4- 96.3), and 62.9 (95% CI 15.7-251.3) respectively. Of the non-transplanted patients who met the KCC, a total of 13 patients were excluded from listing for OLT due to active and resistant alcohol dependence (11 patients) and active intravenous drug abuse (2 patients). A total of 4 patients were listed but subsequently deemed medically unfit to survive OLT, whilst a further two patients showed clear signs of spontaneous recovery and were delisted prior to an organ becoming available. As shown in **table 4.3**, on admission patients who later died or were transplanted were significantly more coagulopathic, had lower serum albumin, and had significantly higher serum creatinine levels compared with spontaneous survivors. A significantly greater proportion of patients who died or were transplanted required multiple organ support, such as RRT, inotropic support, and mechanical ventilation (**table 4.3**).

Variable		Dead/OLT (n=21)	Spontaneous survival (n=79)	*p*
Admission laboratory parameters	Platelets (x10⁹/L)	100 (34-167)	120 (74-160)	0.186
	Sodium (mmol/L)	135 (132-138)	136 (133-138)	0.545
	Creatinine (μmol/L)	174 (118-239)	94 (75-165)	0.006
	ALT (IU/L)	6697 (4278-11920)	9132 (6117-12360)	0.154
	Bilirubin (μmol/L)	76 (56-90)	86 (56-110)	0.369
	Albumin (g/L)	30 (23-35)	34 (31-37)	0.013
	PT (seconds)	80 (53-103)	48 (33-70)	0.002
Developed encephalopathy		21 (100%)	26 (32.9%)	<0.001
Met King's College Criteria		17 (81.0%)	12 (15.0%)	<0.001
RRT		17 (81.0%)	14 (17.5%)	<0.001
ICP monitor		17 (81.0%)	7 (8.9%)	<0.001
Inotropic support		17 (81.0%)	6 (7.6%)	<0.001
Mechanical ventilation		20 (95.2%)	15 (18.8%)	<0.001
Transplanted		3	0	-

Table 4.3 Admission laboratory parameters and clinical outcomes for study population.

ALT, alanine aminotransferase; PT, prothrombin time; RRT, renal replacement therapy; ICP, intracranial pressure

4.5.3 Temporal relationship between overdose and liver injury

The temporal relationship between overdose and serum ALT (as a marker of hepatic necrosis) was determined for each six-hour period post overdose for patients who died or were transplanted (n=21) and for spontaneous survivors (n=79). As shown in **figure 4.1**, there was no significant difference in median peak ALT levels between survivors (10500 (7564-13650) IU/L) and patients who died or were transplanted (8540 (6220-12800) IU/L, p=0.125), whilst at several time points mean ALT levels were significantly *lower* in patients who died compared with survivors. Similarly, the median time from overdose to maximum ALT level was similar in both survivors (66 (54-72) hours) and patients who died/were transplanted (60 (42-72) hours, p=0.276). The AUC was similar for both survivors (650305 IU) and non-survivors (610632 IU, p>0.05).

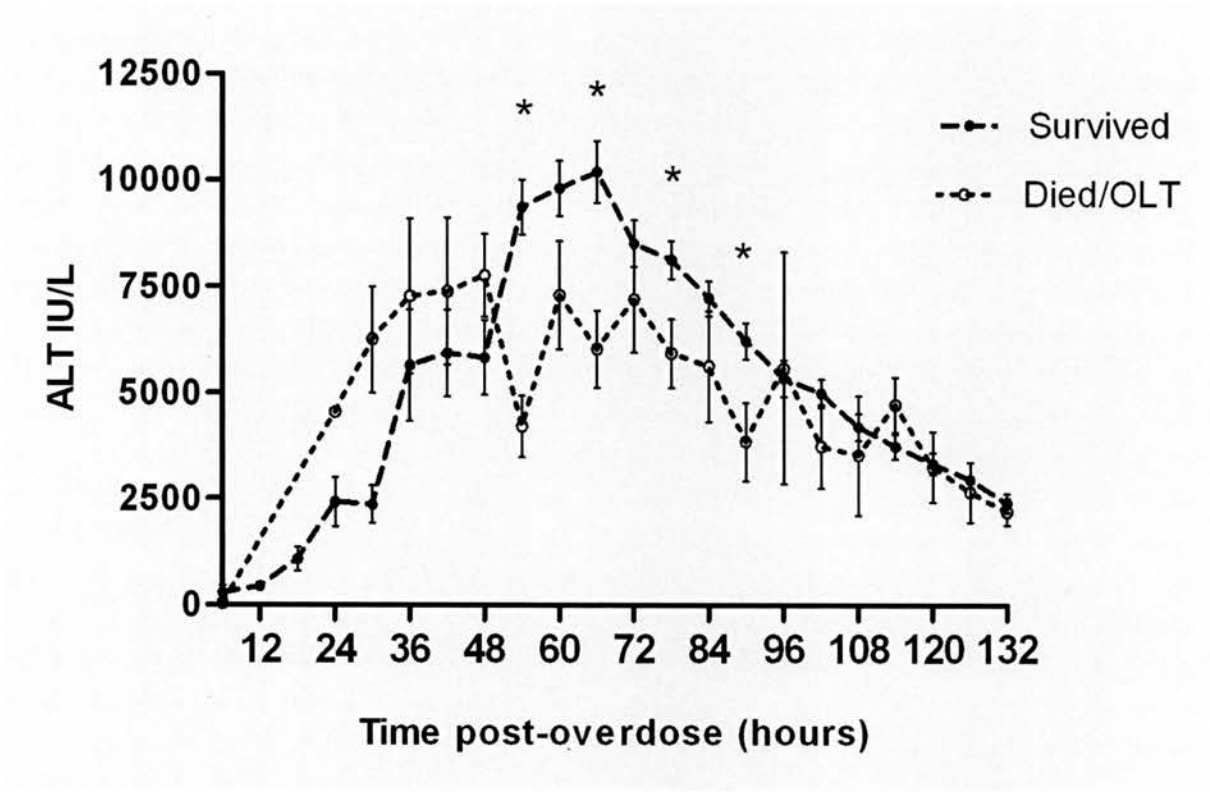

Figure 4.1 The temporal profile of serum alanine aminotransferase (ALT) levels in spontaneous survivors (n=79) and dead/transplanted (n=21) patients following single time point paracetamol overdose.

Data are presented as mean +/- standard error of the mean.

* $p<0.05$ at specific time point (Bonferroni correction applied)

4.5.4 SIRS and temporal relationship to overdose

The relative proportions of patients exhibiting a SIRS response at each 24 hour period post overdose, and the maximum number of SIRS components fulfilled, for the two groups of patients was then explored. These data are displayed in **table 4.4** and **figure 4.2**. By 48 hours post overdose, a total of 27/40 (67.5%) patients had mounted a SIRS response, with a mortality rate in the SIRS group of 44.4%, compared with 7.7% for those patients not mounting a SIRS response (Fisher's p=0.020). By 72 hours post-overdose, a total of 58/85 (68.6%) patients had developed a SIRS during admission, with a mortality rate of 28.8%, compared with a mortality rate of only 3.7% (1/27 patients, Fisher's p=0.007) in patients without a SIRS by 72 hours. By 96 hours, a SIRS had occurred in 70 (70%) patients, with a mortality rate of 30% in this group; compared with a mortality rate of 0% in the 30 patients without the SIRS (Fisher's p=0.001). There were no significant differences in the proportions of male and female patients developing the SIRS by 96 hours post overdose (p=0.149). The sensitivity, specificity, and DORs of the SIRS occurring by each time point post-overdose are shown in **table 4.4**. In total, 74 (74%) of patients developed the SIRS post-overdose, but this occurred significantly earlier following overdose in patients who died (median 43 (28.5-61) hours, n=21) compared with patients who survived (60 (36.5-81) hours, n=53, p=0.05). In contrast, the presence of the SIRS at the time of hospital admission, or at the development of HE, resulted in reduced sensitivity and DORs compared with the values obtained at 48, 72, and 96 hours post-overdose (**table 4.4**).

Time from overdose (hours)	N/deaths	Any SIRS/ deaths	Sensitivity (95% CI)	Specificity (95% CI)	Diagnostic Odds Ratio (95% CI)
48	40/13	27/12	92.3 (64.0- 99.8)	44.4 (25.5- 64.7)	9.6 (1.1- 84.6)
72	85/18	58/17	94.4 (72.7- 99.9)	38.8 (27.1- 51.5)	10.8 (1.4-85.9)
96	100/21	70/21	100.0 (83.9- 100.0)	38.0 (27.3- 49.6)	∞ (3.3-∞)
At SLTU admission	100/21	52/17	81.0 (62.4-92.1)	55.7 (50.8-58.7)	5.3 (1.7-16.5)
At onset of HE	47/21	30/18	85.7 (70.7- 94.6)	53.8 (41.7- 61.0)	7.0 (1.7- 27.6)
Modified KCC	100/21	KCC +ve/ deaths 22/17	81.0 (65.0- 90.7)	93.7 (89.4- 96.3)	62.9 (15.7- 251.3)

Table 4.4 Predictive accuracy of the presence of the Systemic Inflammatory Response Syndrome (SIRS) following single time point paracetamol overdose.

The SIRS was defined as the presence of ≥2 SIRS components in any 24-hour period. The accuracy of the SIRS is compared with the modified King's College Hospital poor prognostic criteria (KCC).

CI, confidence intervals; HE, hepatic encephalopathy; SLTU, Scottish Liver Transplantation Unit

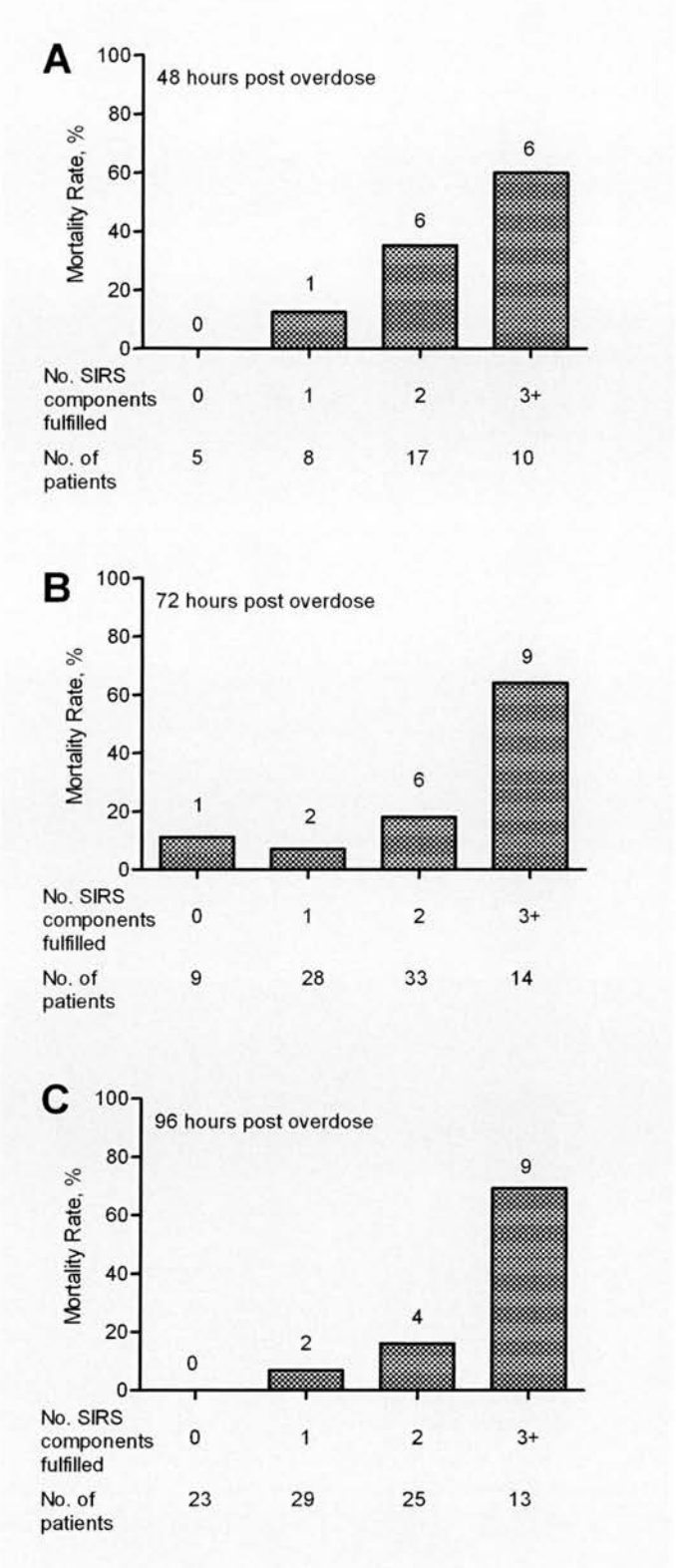

Figure 4.2. Mortality rate in relation to the changes in systemic inflammatory response syndrome (SIRS) score during the first 96 hours following single time point paracetamol overdose.

Numbers above the bars indicate number of deaths.

4.5.5 Temporal relationship between overdose and SOFA score

SOFA scores could be calculated for 99/100 patients. SOFA scores ranged from 1 to 15 by 48 hours post-overdose, 0-17 by 72 hours, and 1-18 by 96 hours post-overdose. Median SOFA score (+/- IQR) was significantly higher in patients who died or were transplanted at each of these three time points (48 hours: 9 (6.25-12.25) vs. 5 (3-7), p=0.009; 72 hours: 12.5 (8.75-17) vs. 5 (3-7), p<0.001; 96 hours: 15 (9-19) vs. 5 (3-8), p<0.001). The number of organ systems failing by both 72 (p=0.0003) and 96 (p<0.0001) hours post-overdose was significantly related to mortality, which ranged from 5.9% for patients without any organ system failure at 72 hours post-overdose to 71.4% for those patients with 3 or more organ system failures at this time point, **figure 4.3**. ROC analysis showed an area under the curve of 0.80 (95% CI 0.65-0.95), 0.90 (95% CI 0.82-0.97), and 0.91 (95% CI 0.85-0.98) at 48, 72, and 96 hours post-overdose respectively, **figure 4.4**. The optimal SOFA score thresholds for discriminating non survivors at these three time points were >4 at 48 hours, >6 at 72 hours, and >7 at 96 hours respectively. A SOFA score >7 at any time during the first 96 hours post overdose also predicted outcome with excellent accuracy, with a significantly higher maximum SOFA score amongst patients who died (15.0 +/- 4.2) compared with spontaneous survivors (6.4 +/- 4.1, p<0.001), **table 4.5**.

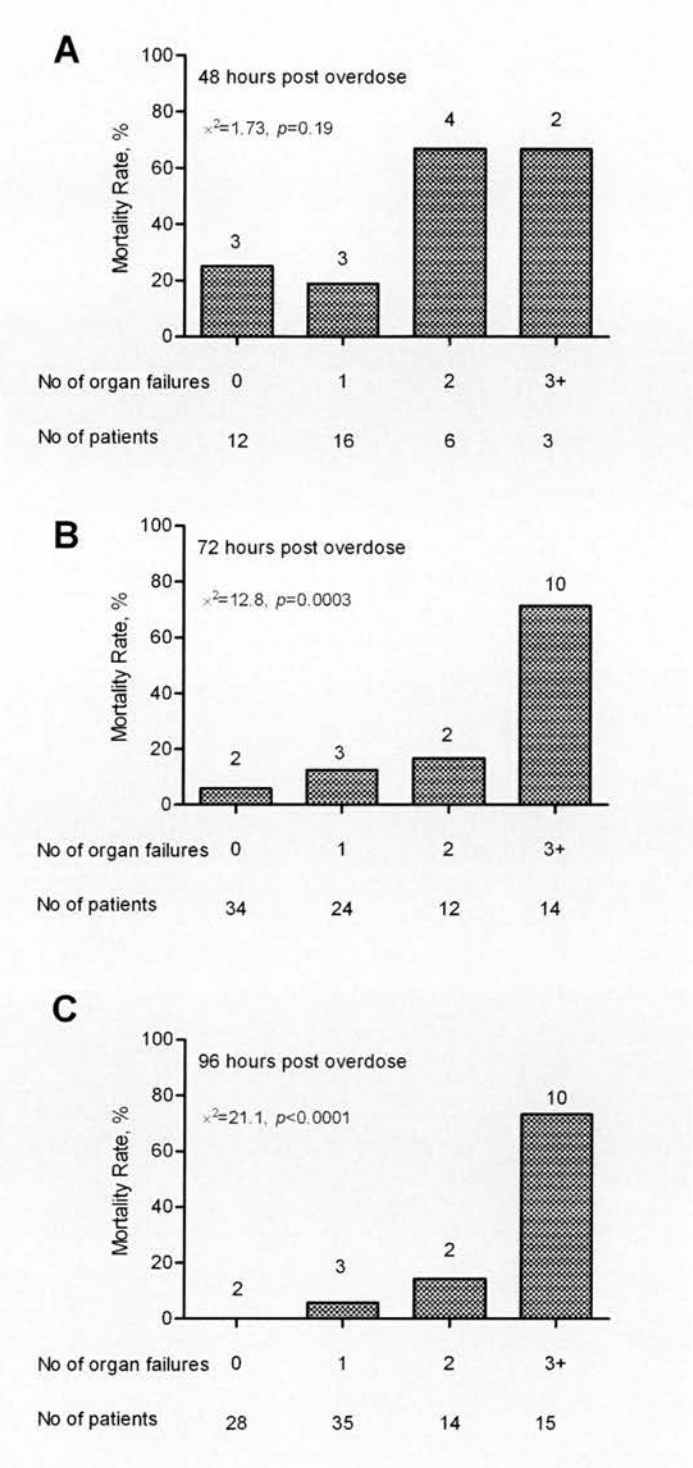

Figure 4.3. Association between mortality rate and the number of organ failures identified by the Sequential Organ Failure Assessment (SOFA) score at (A) 48, (B) 72, and (C) 96 hours following single time point paracetamol overdose.

Numbers above the bars indicate number of deaths.

Death and liver transplantation were considered equivalent.

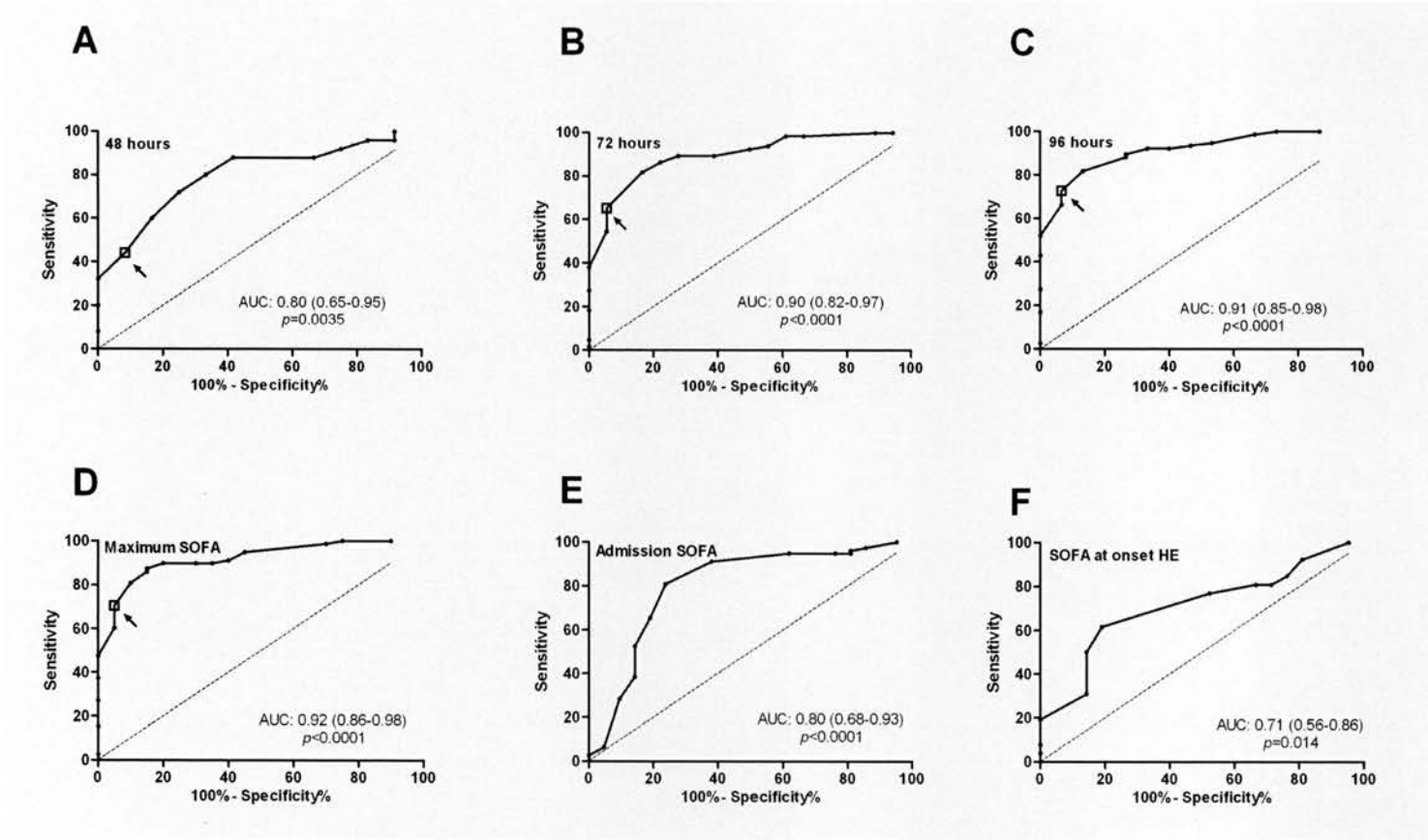

Figure 4.4. Comparisons of the areas under the Receiver Operator Characteristic (ROC) curves for prediction of mortality using the Sequential Organ Failure Assessment (SOFA) score.

The area and 95% confidence interval (CI) are presented in each panel. Data markers are the optimal threshold for each SOFA score that discriminates between survival and non-survival (death or liver transplantation). HE, hepatic encephalopathy

Time from overdose (hours)	Threshold SOFA score	N/ deaths	SOFA >threshold / deaths	Sensitivity (95% CI)	Specificity (95% CI)	Diagnostic Odds Ratio (95% CI)
48	>4	37/12	25/11	91.7 (61.5- 99.8)	44.0 (24.4- 65.1)	8.6 (1.0- 77.5)
72	>6	84/18	40/17	94.4 (72.7- 99.9)	65.2 (52.4- 76.5)	31.8 (4.0-254.2)
96	>7	92/15	35/14	93.3 (68.1- 99.8)	72.7 (61.4- 82.3)	37.3 (4.6-301.8)
Up to 96	>7	98/20	42/19	95.0 (78.5- 99.1)	70.5 (66.3- 71.6)	45.4 (7.2- 278.7)
At SLTU admission	>8 (Schmidt and Larsen 2006)	99/21	12/8	38.1 (23.7- 48.7)	94.9 (91.0- 97.7)	11.4 (3.1- 41.0)
At onset of HE	>12 (Schmidt and Larsen 2006)	47/21	9/5	23.8 (12.2- 34.2)	84.6 (75.3- 93.0)	1.7 (0.4- 6.9)
Modified KCC	N/A	100/21	KCC +ve/ deaths 22/17	81.0 (65.0- 90.7)	93.7 (89.4- 96.3)	62.9 (15.7- 251.3)

Table 4.5. Predictive accuracy of the Sequential Organ Failure Assessment (SOFA) score following single time point paracetamol overdose. Optimal thresholds for predicting death or liver transplantation were derived from receiver operator characteristic analysis or previously reported studies. The accuracy of the SOFA score is compared with the modified King's College Hospital poor prognostic criteria (KCC). CI, confidence intervals; HE, hepatic encephalopathy; SLTU, Scottish Liver Transplantation Unit

4.5.6. Evaluation of previously reported SOFA thresholds

A previous report also evaluated SOFA scores following paracetamol overdose, and reported that a SOFA score >8 early after admission, or >12 at the onset of HE, were the most discriminatory thresholds for determining prognosis.(Schmidt and Larsen 2006) Evaluation of these thresholds was therefore performed in this cohort, with the results shown in **table 4.5** and **figure 4.4**. At admission, the area under the ROC for SOFA was 0.80 (95% CI 0.68-0.93), but using a threshold of >8 to predict a poor prognosis resulted in a low sensitivity of 38.1 (95% CI 23.7- 48.7). The area under the ROC for SOFA at the onset of HE (n=47) was 0.71 (95% CI 0.56- 0.86), with a low sensitivity of 23.8 (95% CI 12.2- 34.2) when using a discriminatory threshold of >12.

4.5.7 Development of the modified SOFA score

Univariate analysis of the individual components of the SOFA score at 72 and 96 hours post-overdose was performed to identify those components which best predicted an adverse outcome (**table 4.6**). All the organ failure components except the hepatic failure element were strongly associated with mortality, so a modified SOFA score, which excluded points for hepatic failure, was computed. ROC analysis of this modified SOFA score showed an area under the curve of 0.81 (95% CI 0.67-0.95), 0.90 (95% CI 0.84-0.97), and 0.91 (95% CI 0.84-0.98) at 48, 72, and 96 hours post-overdose respectively. A modified SOFA threshold score of >3 at 48 hours, and >4 at 72 hours, improved upon the specificity of the original SOFA score at both these time points without affecting sensitivity. A modified SOFA threshold score of >5 at any point during the first 96 hours following overdose was highly predictive of death or OLT, with a sensitivity of 95.0 (95% CI 78.7-99.1), specificity of 76.9 (95% CI 72.8-78.0), and negative predictive value of 98.4 (95% CI 93.0-99.7) (**table 4.7**).

Variable		72 hour univariate OR (95% CI) n=84	*p*	96 hour univariate OR (95% CI) n=92	*p*
Sex (male)		0.90 (0.33-2.50)	1.00	1.58 (0.59-4.24)	0.360
Age (>40 years)		0.77 (0.25-2.36)	0.78	1.03 (0.99-1.07)	0.102
SIRS (yes/no)		10.52 (1.66-64.80)	0.009	∞ (3.26- ∞)	<0.001
SOFA (per 1 additional unit)		1.42 (1.22-1.65)	<0.001	1.39 (1.21-1.60)	<0.001
SOFA organ failures (≥3 points per component)	**Cardiovascular failure**	22.75 (4.13-125.37)	<0.001	49.33 (10.20-238.64)	<0.001
	Respiratory failure	24.00 (5.36-107.47)	<0.001	152.00 (16.09-1436)	<0.001
	Neurologic failure	9.21 (2.79-30.39)	0.001	40.00 (9.06-176.54)	<0.001
	Renal failure	5.16 (1.65-16.10)	0.005	4.03 (1.28-12.72)	0.018
	Haematologic failure	22.50 (5.56-91.10)	<0.001	25.00 (4.37-142.90)	<0.001
	Hepatic failure	0.55 (0.16-1.88)	0.255	1.06 (0.35-3.20)	0.574

Table 4.6. Risk factors for inhospital mortality at 72 and 96 hours post-paracetamol overdose: Results of univariate analysis

Time from overdose (hours)	Threshold SOFA score	N/ deaths	SOFA >threshold / deaths	Sensitivity (95% CI)	Specificity (95% CI)	Diagnostic Odds Ratio (95% CI)
48	>4	37/12	25/11	91.7 (61.5- 99.8)	44.0 (24.4- 65.1)	8.6 (1.0- 77.5)
72	>6	84/18	40/17	94.4 (72.7- 99.9)	65.2 (52.4- 76.5)	31.8 (4.0-254.2)
96	>7	92/15	35/14	93.3 (68.1- 99.8)	72.7 (61.4- 82.3)	37.3 (4.6-301.8)
Up to 96	>7	98/20	42/19	95.0 (78.5- 99.1)	70.5 (66.3- 71.6)	45.4 (7.2- 278.7)

Table 4.7. Predictive accuracy of the modified SOFA score following single time point paracetamol overdose. Optimal thresholds for predicting death or liver transplantation were derived from receiver operator characteristic analysis.

4.5.8 Association between SIRS and SOFA scores

We then explored whether those patients with high SOFA scores also manifested a SIRS response. The presence of the SIRS appeared crucial to the development of extrahepatic organ failure, with 32/63 (50.8%) patients with the SIRS by 96 hours post-overdose having developed at least one extrahepatic organ failure, with 13/32 (40.6%) of these cases having ≥3 extrahepatic organ failures. In contrast, only 4/29 (13.8%) patients without the SIRS developed one or more extrahepatic organ failures by 96 hours post-overdose. The median (+/- IQR) SOFA score in those patients who manifested a SIRS response was significantly higher (8 (5-15)) compared with those patients with no SIRS at that point (5 (3-6.25), p=0.0011). There was a strong correlation between these two variables (Spearman's r=0.474, p<0.0001), as shown in **figure 4.5**, with those patients who died tending to have both a SIRS response and a higher SOFA score than spontaneous survivors.

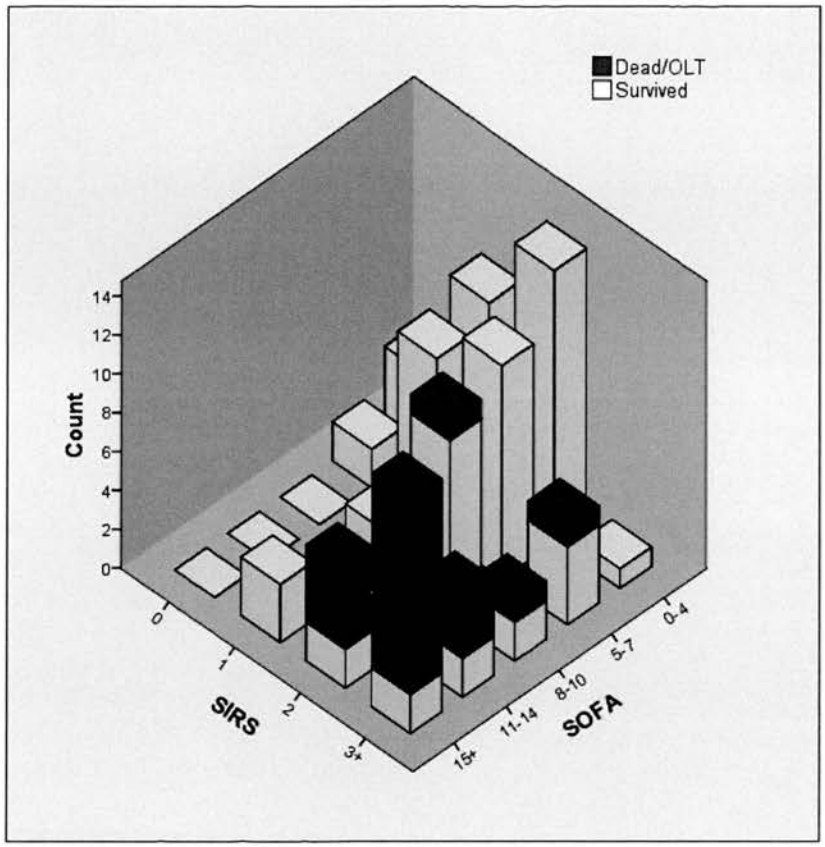

Figure 4.5. Association between Sequential Organ Failure Assessment (SOFA) scores and the number of Systemic Inflammatory Response Syndrome (SIRS) components fulfilled by 96 hours following single time point paracetamol overdose.

4.5.9 Prospective validation of multiorgan failure scores

The prognostic accuracy of the SIRS and SOFA thresholds outlined above was then examined in a prospectively collected cohort of paracetamol-induced acute liver injury patients admitted to the Royal Infirmary of Edinburgh between March 2009 and July 2010. A total of 38 patients were included, and their demographic details, admission laboratory parameters, and clinical outcomes are outlined in **table 4.8**. All 7 patients who died or underwent emergency OLT manifested a SIRS response, had a SOFA score >7, and had a modified SOFA >5 within 96 hours of overdose. The specificity of the two SOFA predictors was 77.4 (95% CI 70.5-77.4) and the specificity of the SIRS was 74.2 (95% CI 67.2-74.2).

Variable (n= 38)		Value
Sex (male/female)		16/22 (42.1%/57.9%)
Age (years)		35 (26.5-44.5)
Received NAC in referring hospital		38 (100%)
Admission laboratory parameters	WCC (x10⁹/L)	8.35 (6.45-11.38)
	Platelets (x10⁹/L)	122 (71-192)
	Creatinine (umol/L)	117 (61-241)
	ALT (IU/L)	5198 (2974-7978)
	Bilirubin (umol/L)	81 (32-113)
	PT (seconds)	41 (29-73)
Ever encephalopathic (ALF) Grade 3-4 HE encephalopathy		18 (47.4%) 12 (31.6%)
Mechanical ventilation		13 (34.2%)
KCC met		9 (23.7%)
SIRS response by 96 hours post-overdose		15 (39.5%)
SOFA score >7 by 96 hours post-overdose		14 (36.8%)
Modified SOFA score >7 by 96 hours post-overdose		14 (36.8%)
Overall outcome Transplanted Spontaneous survival Died without transplantation		 4 (10.5%) 31 (81.6%) 3 (7.9%)

Table 4.8. Validation cohort of 38 single time point paracetamol overdoses

4.6 Discussion

This chapter has explored the temporal relationship between paracetamol overdose and the development of systemic inflammation and organ failure and further confirms that there is little, if any, relationship between the degree of initial liver insult (in terms of ingested paracetamol dose) and subsequent outcome,(Gregory and others 2010) and also confirms that the magnitude of the serum ALT rise (as a marker of hepatic injury) bears no prognostic significance in this condition. In contrast, development of the SIRS and subsequent organ failure are both strongly associated with adverse clinical outcomes, and both SIRS and SOFA scores are highly sensitive markers of poor outcome following paracetamol overdose. Due to the high negative predictive values of both SIRS and SOFA, these scores may have a future role as 'gatekeepers' when considering patient transfer to tertiary liver centres. The risk of death was less than 2% in those acute severe liver injury patients without a SOFA score >7 during the first 96 hours following paracetamol overdose.

Predicting individual prognosis accurately following paracetamol overdose is fraught with difficulty.(O'Grady 2005) For a predictive model to be clinically useful, it should be easy to use, accurate, reproducible, and accepted by clinical staff. Both SOFA and SIRS scores are dynamic tests, which can be rapidly recalculated throughout admission, and are attractive as prognostic indicators due to their widespread use in intensive care units and their ease of use. Recently, the SOFA score was demonstrated to outperform liver-specific prognostic scores in predicting outcome amongst a cohort of critically ill chronic liver disease patients.(Das and others 2010) Although neither SIRS nor SOFA were originally developed to predict outcome, the high sensitivity of these scores makes them attractive as a means of identifying all those high-risk paracetamol overdose patients who might die without transplant, although their relatively lower specificity suggests that they should not replace the KCC as definitive listing criteria. Additionally, there are some specific limitations of both SOFA and SIRS scores that should be addressed. The SIRS does not incorporate a hepatic failure component at all, whilst the hepatic failure component (serum bilirubin) of the SOFA score lacks specificity or the ability to differentiate acute from chronic hepatic dysfunction. Indeed, of all the 6 organ failure components combined in the SOFA score, only the hepatic failure element was not associated with an increased risk of death, a finding in keeping with several previous studies of this scoring system.(Janssens U. and others 2000)

The principal strength of this study lies in its novel evaluation of SIRS and SOFA scores in relation to the timing of overdose, rather than in relation to the time of admission or the onset of HE. The initial symptoms of ALF are often vague, and as a result there may be a significant delay between the overdose and subsequent presentation to medical services. Additionally, some patients present as a result of the psychological consequences of their overdose, rather than as a result of physical morbidity. Attempting to apply a prognostic score at the time of admission is therefore considerably hindered by patient heterogeneity. Equally, accurately applying a prognostic score at the onset of HE may be problematic since the presence or absence of HE is subjective, and subtle encephalopathy may be wrongly interpreted as resulting from alcohol or narcotic withdrawal. In an attempt to minimise clinical heterogeneity only single time point overdoses were selected, with the evolution of systemic inflammation and organ failure examined in relation to this fixed time point. Using this approach, it can be seen that the prognostic accuracy of both SIRS and SOFA scores are enhanced compared with previous reports which applied these markers at either the time of hospital admission or the onset of HE.(Cholongitas and others 2006b; Schmidt and Larsen 2006) Clearly, this approach is dependent upon the temporal accuracy of the patients' overdose history, but previous studies have suggested that the patient history is usually reliable following paracetamol overdose.(Waring and others 2008) Heterogeneity is further reduced by the single centre nature of this study, where both criteria for patient admission and clinical management protocols have remained largely unchanged during the time course of the study.

However, this retrospective study has several limitations. Clearly, the results of this study are not directly applicable to patients who have taken a staggered overdose, an increasingly recognised subtype of paracetamol overdose (**see chapter 3**). Although this study has examined the applicability of multiorgan failure scores in relation to time from overdose, patients access treatment at different time points in their clinical evolution, which may influence the development of the SIRS or organ failure. A further caveat is that patients were transferred to the SLTU from a number of surrounding hospitals, each of which may have had different management protocols, clinical expertise, and referral thresholds following paracetamol overdose. Whilst the SLTU is the single referral point for all severe paracetamol overdoses in Scotland, we recognise the possibility of selection bias and that some patients

with severe organ failure secondary to paracetamol overdose may not have been transferred to the SLTU. It is also recognised that not all of the patients in this study developed HE, and therefore ALF. However, predicting the development of HE can be equally problematic, and the mere absence of HE following severe acute liver injury does not preclude the development of other complications such as acute kidney injury.(Pakravan and others 2009)

A further potential application for SIRS and SOFA scores may be as 'gatekeepers' to more accurately determine requirements for tertiary level care amongst patients with paracetamol-induced acute liver injury. In the SLTU, transfer criteria are based broadly upon those used in a previous King's College study and upon guidelines previously published by the British Society of Gastroenterology.(Devlin and O'Grady 1999) These guidelines recognise that "A significant proportion of cases will make an unremarkable recovery without intensive measures but this level of unnecessary transfer is justified if the potential for transplantation, in what is often a narrow window of time, is to be realised". Due to the rapid progression of hyperacute ALF (including aetiologies such as paracetamol and ecstasy) and the risks involved in transferring patients with HE or with incipient cerebral oedema, up to 50% of UK liver centre patients never develop HE. Developing highly sensitive markers to identify high-risk hyperacute patients at an earlier time point, prior to HE developing, could aid safe transfer, and equally the high negative predictive value of the multiorgan scores outlined in this study could be used to safely manage patients without the need for transfer. The modified SOFA score outlined above might be particularly suited to this gatekeeper role given the improved specificity of this score compared with the original SOFA, and the increasing recognition of the importance of extrahepatic organ dysfunction following acute liver injury.(Parekh and others 2007; Wu and others 2010)

In conclusion, this retrospective cohort study of 100 severe acute paracetamol-induced acute liver injury patients has evaluated the incidence and temporal kinetics of both the SIRS and SOFA scores following the paracetamol overdose, and has demonstrated that these markers are highly sensitive at identifying patients with a poor prognosis. The high negative predictive value of these markers was validated in a prospective cohort. Both the SIRS and

SOFA scores deserve further evaluation and external validation as potential gatekeepers to improve clinical decision making models following paracetamol overdose.

Chapter 5. Increased circulating heavy ferritin and extreme hyperferritinaemia in paracetamol-induced human acute liver injury

5.1 Introduction

5.2 Methods

5.2.1 Retrospective cohort study.

5.2.2 Prospective cohort study

5.2.3 Serum ferritin and cytokine measurements

5.2.4 Histological analysis

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5.4.2 Extreme hyperferritinaemia is common in paracetamol-induced acute liver injury

5.4.3 Disordered indices of iron metabolism are common in acute liver injury.

5.4.4 Hyperferritinaemia in acute liver injury is not associated with significant hepatic iron overload.

5.4.5 Correlation of serum ferritin with liver injury, function, and outcome

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5.4.7 Elevated serum ferritin in acute liver injury is associated with significant levels of circulating H-ferritin

5.5 Discussion

Chapter 5. Increased circulating heavy ferritin and extreme hyperferritinaemia in paracetamol-induced human acute liver injury

5.1 Introduction

The previous chapter highlighted that a SIRS response is a frequent phenomenon amongst patients who go on to develop paracetamol-induced ALF. It is well recognised that paracetamol-induced ALF is associated with disordered functioning of the cellular components of the innate immune system, in particular monocyte-macrophages,(Holt, Cheng, Ju 2008) dendritic cells,(Chen and others 2009) and neutrophils,(Liu and others 2006), but a greater understanding of the pathophysiology of the SIRS response could allow earlier identification of patients at risk of developing systemic complications following paracetamol overdose. Additionally, a greater pathophysiological understanding of the SIRS response could identify alternative biomarkers of poor prognosis and thereby improve outcomes by accelerating use of multidisciplinary supportive interventions and hastening patient transfer to tertiary liver centres.

Ferritin is a ubquitous 24-subunit iron-binding cytosolic protein expressed in most tissues which is capable of sequestering up to 4500 Fe (III) atoms. Ferritin forms a spherical shell consisting of variable amounts of heavy (H) and light (L) chain subunits, with H-rich tissue ferritins found predominantly in heart and kidney, and L-rich forms predominating in liver and spleen.(Torti and Torti 2002) In addition to tissue ferritins, small amounts of ferritin are also found in serum, and are of great clinical value in the diagnosis and monitoring of iron deficiency anaemia and iron overload states such as hemochromatosis.(Knovich and others 2009) Although elevated serum ferritin is characteristic of hemochromatosis, non-specific increases in circulating ferritin also occur in diverse inflammatory conditions such as atherosclerosis,(You and Wang 2005) malignancy,(Hazard and Drysdale 1977), chronic kidney disease,(Kalantar-Zadeh, Kalantar-Zadeh, Lee 2006) and autoimmune disease.(Recalcati and others 2008) Particularly remarkable elevations of serum ferritin are seen in Still's disease,(Evensen, Swaak, Nossent 2007) and haemophagocytosis.(Emmenegger and others 2005) A recent report suggested that serum ferritin may be an additional prognostic marker to the MELD score in patients awaiting OLT.(Walker and others 2010) Intense interest has focused upon a potential immunomodulatory role for H-ferritin, due to

the immunosuppressive effects of ferritin upon both lymphoid (Harada and others 1987) and myeloid (Broxmeyer and others 1989) cells, possibly through induction of interleukin (IL)-10 production by lymphocytes.(Gray and others 2001) Historically, hyperferritinaemia was noted to reflect the degree of hepatocellular damage more closely than serum transaminases in a cohort of 25 patients admitted following paracetamol overdose,(Eastham, Bell, Douglas 1976) but ferritin levels have not been prospectively measured in paracetamol-induced ALF patients. It was hypothesised that serum ferritin would be elevated in patients following acute liver injury and that this might serve as an early prognostic marker.

5.2 Methods

5.2.1 Retrospective cohort study.

Retrospective analysis of the SLTU acute liver injury database was performed to identify all patients who had an admission serum ferritin and/or iron studies performed as part of a liver aetiology screen between January 2002 and December 2008.

5.2.2 Prospective cohort study

The study was prospectively approved by the Scotland 'A' Research Ethics Committee. Informed consent or assent was obtained from all patients or the patient's nominated next of kin prior to study inclusion. A total of 47 adult (>16 years) patients admitted to the Royal Infirmary of Edinburgh with ALF or acute liver injury were entered into the study. Acute liver injury and ALF were defined as described in previous chapters. Admission laboratory and clinical parameters, including encephalopathy grade, mean arterial pressure, WCC, platelet count, INR, serum electrolytes, serum bilirubin, and serum ALT were prospectively recorded in a dedicated database. The severity of hepatic and multiorgan dysfunction was assessed according to the modified KCC and the APACHE II score respectively.(Bernal and others 2002; Knaus and others 1985) Additionally, SIRS and SOFA scores were calculated throughout admission. The requirement for, or development of, any of the following clinical parameters during admission was documented: mechanical ventilation, treatment for increased intracranial pressure, inotropic support, renal replacement therapy, or hypoglycaemia.

5.2.3 Serum ferritin and cytokine measurements

Peripheral serum and plasma samples were collected sequentially in serum gel and potassium- ethylenediaminetetraacetic acid containing plastic tubes (Sarstedt, Leicester, UK) and centrifuged at 1000g for 15 minutes at 4ºC within one hour following collection. Plasma and serum aliquots were immediately stored in polypropylene tubes at -80ºC until analysis. Samples were obtained from patients on admission, and then daily for the first five days, or longer if clinically indicated. Serum ferritin was assayed on an automated immunoassay system using a chemiluminometric endpoint with an acridinium ester - labelled polyclonal goat anti-ferritin antibody and a monoclonal mouse anti-ferritin capture antibody (Advia Centaur; Siemens Healthcare Diagnostics Ltd., Camberley, UK). Where necessary, samples

were diluted and then reassayed. All samples were analysed in a blinded fashion. The precision of this immunoassay was < 5% over the range of concentrations measured.

Serum IL-6, IL-8, and IL-10 measurements were performed using a cytometric bead array kit and software (BD Biosciences, San Jose, CA.) performed according to the manufacturers instructions. Briefly, serum-enhancement treated capture beads, test samples and standards were added to a 96-well plate and agitated on a plate shaker. Following incubation together for 1.5 hours, the supernatant was aspirated and phycoerythrin detection reagent added. Following a further 1.5 hour incubation, cytokine analysis was performed on a FACSArray® flow cytometer (BD Biosciences, San Jose, CA.).

5.2.4 Histological analysis

Tissue samples obtained from the hepatic explant were fixed in buffered formalin and embedded in paraffin. Liver sections were stained with Perls' Prussian blue iron stain and the distribution of stainable iron (hepatocytes, bile duct cells, and Kupffer's cells) was recorded by a pathologist (Dr C Bellamy) in blinded fashion.

5.2.5 Western blotting for H- and L-ferritin

Snap frozen sections of previously archived healthy human liver were defrosted on ice, homogenised with ice-cold lysis buffer, and subsequently heated to 100°C with Laemmli buffer in a 1:2 ratio for 5 minutes prior to loading on 10.0% sodium dodecyl sulfate–polyacrylamide gels. Similarly, serum samples were diluted 1:2 with Laemmli buffer, heated to 100°C for 5 minutes and 5μL of each diluted boiled sample loaded on to duplicate gels to provide a qualitative assessment of the presence or absence of ferritin isoforms. Following electrophoresis, proteins were transferred to nitrocellulose membranes which were subsequently blocked with 1 x Tris-buffered saline (TBS)/0.1% Tween-20/5% non-fat milk powder for 2 hours prior to overnight incubation with either rabbit anti-H or rabbit anti-L ferritin monoclonal antibodies (Abcam, Cambridge, UK). After washing with TBS/0.1% Tween-20, the membranes were incubated for 1 hour at 4°C with horseradish peroxidase labelled goat anti-rabbit secondary antibody (DakoCytomation, Carpinteria, CA.), and the proteins identified using a chemiluminescent substrate staining kit (Pierce Biotechnology, Rockford, IL.) and subsequent 10-second exposure to a photographic plate.

5.3 Statistical Analysis

All data were prospectively recorded in the SLTU ALF database. Statistical analysis was performed using SPSS software (SPSS 16.0, Chicago IL, USA) and Graphpad Prism (GraphPad Software Inc., La Jolla, CA). Data values are presented as median +/- IQR or percentages unless otherwise stated. Continuous data were compared using the student's t-test or Mann-Whitney U test for non-normally distributed variables, and by analysis of variance or the Kruskal-Wallis test when three or more groups were compared. Categorical data were analysed using Chi-squared tests or Fishers exact test. The Bonferroni method was used to adjust *p*-values to account for multiple comparisons. A two-sided p value of <0.05 was considered statistically significant.

5.4 **Results**

5.4.1 Patient characteristics and outcomes

The initial retrospective analysis included 124 patients with acute liver injury in whom serum ferritin and iron indices had been measured on admission to the SLTU. Paracetamol overdose (POD) was the most common aetiology in this cohort; POD 71 (57.2%), non-POD 53 (42.7%). The demographics and admission laboratory parameters of these two patient groups are outlined in **table 5.1**. Non-POD patients were significantly older and had higher admission bilirubin levels, but lower serum ALT values, compared with POD patients. POD patients were significantly more coagulopathic than non-POD patients. Most (100, 80.6%) patients were transferred from other health boards in Scotland with the remaining 24 patients transferred from local hospitals or other wards within the Royal Infirmary of Edinburgh. Most of the overdoses were taken in a suicidal attempt (51 patients, 71.8%). All patients with POD received NAC prior to, or on admission to the SLTU. A total of 65 (52.4%) patients (42 POD, 23 non-POD) never developed HE and were classified as having acute liver injury rather than ALF, and all of these patients survived. In the 59 patients with ALF (POD 29, non-POD 30) 25 (42.4%) recovered spontaneously (POD 16/29, 55.2%; non-POD 9/30, 30.0%) and 18 (30.5%) patients underwent emergency LT (POD 5/29, 17.2%; non-POD 13/30, 43.3%). Comparison of the clinical details of patients in whom ferritin was measured compared with those with no ferritin measurement are presented in **table 5.2**.

Variable	Paracetamol (n=71)	Non-paracetamol # (n=53)
Age (years)	38 (27-46) *	51 (38-58)
Sex (M/F)	42 (59.1%) / 29 (40.7%)	20 (37.6%) / 33 (62.4%)
Leucocyte count (x10^9/L) (NR = 4-11 x10^9/L)	10.7 (7.6-12.8)	9.4 (7.0-11.8)
Prothrombin time (seconds) (NR 12-14 seconds)	52 (36-71) *	21.0 (16-39)
Sodium (mmol/L) (NR 135-145 mmol/L)	135 (133-139)	134 (130-138)
Creatinine (mmol/L) (NR 60-110 mmol/L)	120 (82-218)	90 (80-170)
Bilirubin (μmol/L) (NR 3-17 μmol/L)	90 (63-129) *	356 (140-462)
ALT (IU/L) (NR 7-50 IU/L)	7078 (3988-11480) *	954 (305-2123)
Iron (NR = 14-32μmol/L)	37 (25-47) * (Range 3-78)	21 (11-38) (Range 3-52)
Transferrin (NR = 2-4g/L)	1.68 (1.39-1.99) (Range 0.78-2.91)	1.68 (1.18-1.92) (Range 0.56-3.03)
Transferrin saturation (%)	90 (74-101) * (Range 5-190)	72 (25-89) (Range 5-100)
Ferritin (NR = 20-300μg/L)	11121 (2068-32915) * (Range 48-114004)	1290 (469-5320) (Range 52-61500)

Table 5.1 Demographic and admission laboratory data for paracetamol and non-paracetamol acute liver injury patients (retrospective cohort).

*= $p<0.05$; NR, normal range Data are presented as median +/- IQR unless otherwise indicated

53 patients with non-paracetamol aetiologies include idiosyncratic drug reactions (n=12), non A-E hepatitis (n=9), autoimmune hepatitis (n=9), ischaemic hepatitis (n=8), hepatitis B infection (n=5), malignancy (n=3), Budd-Chiari syndrome (n=2), sepsis (n=2), others = 3.

Admission characteristics		No Ferritin measurement n=842 Median (IQR) /N (%)	Ferritin measured n=124 Median (IQR) /N (%)	*p*
Sex (male/female)		368/474 (43.7%/56.3%)	62/62 (50%)	n.s.
Age (years)		42 (32-52)	42 (32-54)	n.s.
Paracetamol cases		571/842 (67.8%)	71/124 (57.3%)	0.020
Single overdose		424/571 (74.3%)	43/71 (60.6%)	0.023
Admission laboratory characteristics	Platelets (x10^9/L) (NR 150-400 x10^9/L)	131 (78-185)	141 (100-201)	n.s.
	Creatinine (mmol/L) (NR 60-110 mmol/L)	132 (83-243)	109 (80-201)	<0.05
	ALT (IU/L) (NR 7-50 IU/L)	5322 (1643-9617)	3363 (1053-8300)	n.s.
	Bilirubin (μmol/L) (NR 3-17 μmol/L)	92 (60-139)	112 (67-286)	<0.05
	Prothrombin time (seconds) (NR 12-14 seconds)	42 (28-63)	40 (23-65)	n.s.

Table 5.2 Clinical characteristics of ferritin cohort compared with other patients admitted with acute liver injury.

5.4.2 Extreme hyperferritinaemia is common in paracetamol-induced acute liver injury

Elevated serum ferritin (i.e. serum ferritin >300μg/L) was common in patients (109/124 patients, 87.9%) with acute liver injury; and increased serum ferritin levels were more frequent in the POD (66/71 patients, 93.0%) compared with the non-POD cohort (43/53 patients, 81.1%, p=0.046). The absolute values for serum ferritin were also significantly higher in patients with paracetamol-induced acute liver injury compared with non-paracetamol group (**table 5.1, figure 5.1**). In addition, extreme elevations of serum ferritin (> 10 000μg/L) were more common in the POD group (36/71, 50.7% patients ferritin > 10 000μg/L; 2/71, 2.8% patients ferritin > 100 000μg/L) compared with the non-POD group (5/53, 9.4% patients ferritin > 10 000μg/L, no patient ferritin > 100 000μg/L, p<0.001, Fishers exact test for ferritin > 10 000μg/L).

5.4.3 Disordered indices of iron metabolism are common in acute liver injury.

In view of the extreme elevation of serum ferritin in patients with acute liver injury analyses of serum transferrin, iron, and transferrin saturations were performed in the retrospective cohort where these had been measured (**figure 5.1**). Elevated serum iron (i.e. serum iron > 32μmol/L; 54/109 patients, 49.5%) was frequently observed in patients with acute liver injury, and was more common in POD patients (POD 40/65, 61.5%; non-POD, 14/44, 31.8%, p=0.002, Fishers exact test) . In contrast, transferrin levels were frequently reduced (serum transferrin <2g/L) in patients with acute liver injury (81/104 patients, 77.9%). Reduced transferrin concentrations (POD, 48/63 (76.2%) patients; non-POD, 33/41 (80.5%) patients, p=0.606) were similarly observed in both groups. Comparing the absolute values for these variables revealed significantly elevated iron and transferrin saturation in patients with paracetamol-induced acute liver injury compared with the non-paracetamol group **(table 5.1, figure 5.1)**.

Anaemia can occur relatively early in the clinical course of critically ill patients and is characterised by reduced serum iron, transferrin and transferrin saturation and mildly increased serum ferritin concentrations.(Walsh and Saleh 2006) Twenty three (18.7%) patients in the retrospective cohort had haemoglobin levels <100g/L on admission (POD 13, 18.3%; non-POD 10, 18.9%, n.s.). In these patients low transferrin (<2g/L; 16/21 patients, 76.2%) and increased ferritin (>300μg/L; 18/23 patients, 78.3%) were relatively common but

low serum iron (<14 μmol/L; 9/22 patients, 40.9%) and transferrin saturations (<15%; 3/21, 14.3%) were less frequently observed, suggesting the changes in iron indices in ALF are distinct from those reported in critically ill patients with anaemia.

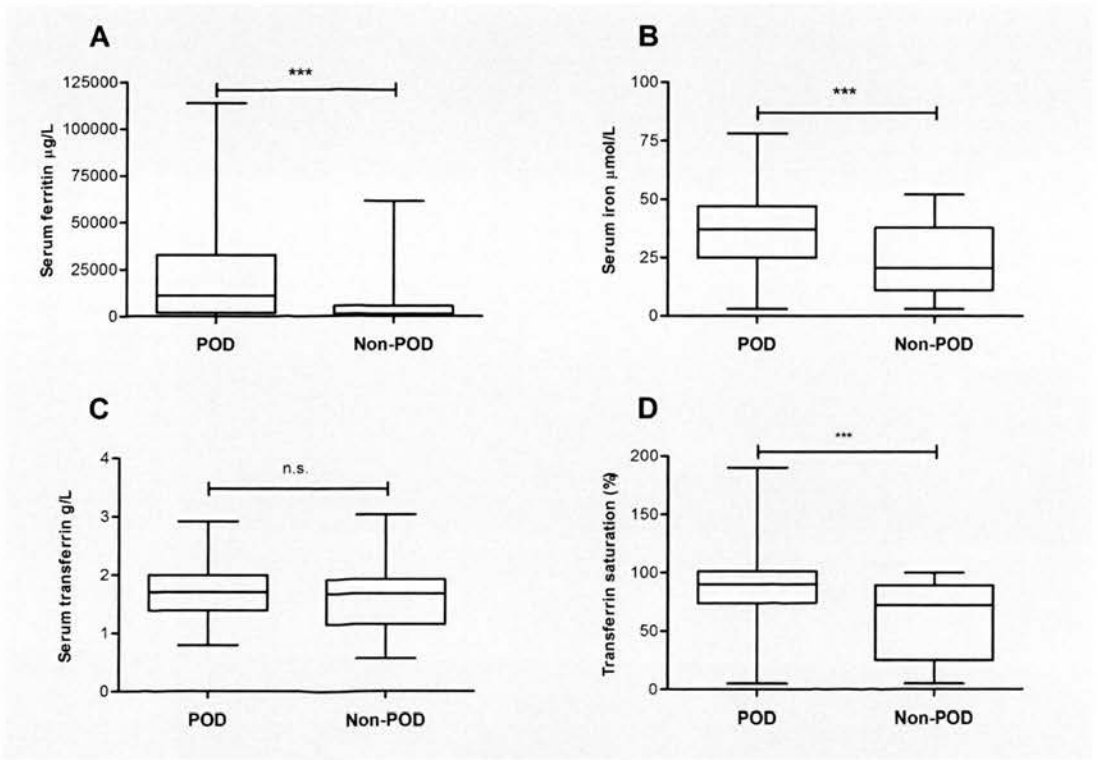

Figure 5.1 Admission serum ferritin and other iron indices following acute liver injury.

Retrospective analysis of SLTU ALF database parameters for serum (A) ferritin, (B) iron, (C) transferrin and (D) transferrin saturation. Box and whiskers plots represent median, quartiles, and extreme data values. *** $p<0.001$, Mann-Whitney U test; n.s. not significant

POD, paracetamol overdose (n=71); non-POD (n=53)

5.4.4. Hyperferritinaemia in acute liver injury is not associated with significant hepatic iron overload

Elevated serum ferritin and transferrin saturation are characteristic features of hemochromatosis. Hepatic tissue removed during OLT was therefore examined for significant iron overload. 18 patients underwent OLT in the retrospective cohort (5 POD, 13 non-POD). None of the explanted cases exhibited significant iron accumulation.

5.4.5 Correlation of serum ferritin with liver injury, function, and outcome

The initial analysis above revealed significant differences in serum ferritin between patients with paracetamol and non-paracetamol induced acute liver injury. In addition, different prognostic criteria are utilised in these two aetiological groups.(O'Grady and others 1989) Analysis of the relationship between prognostic factors in paracetamol and non-paracetamol aetiologies was therefore performed separately. It was considered that the elevated ferritin levels in paracetamol patients might simply reflect underlying hepatic necrosis; however, there was no significant correlation between admission serum ALT and ferritin levels (Spearman's r=0.172, p=0.152). However, there was a weak correlation between ferritin and hepatic dysfunction, as assessed by PT (Spearman's r=0.255, p=0.033). Serum ferritin was significantly higher in paracetamol patients who died or were transplanted (38580 (6702-54533) μg/L, n=13) compared with paracetamol patients who survived spontaneously (8030 (1700-28403) μg/L, n=58, p=0.011, Mann-Whitney U test). Similarly, admission ferritin levels were significantly higher in those paracetamol patients who developed HE, and therefore ALF, at some point during admission (18160 (3880-47305) μg/L, n=29) compared with paracetamol acute liver injury patients (7725 (1566-24548) μg/L, n=42, p=0.038, Mann-Whitney U test). Serum ferritin levels were similar in paracetamol patients with elevated creatinine (creatinine ≥120μmol/L: 19777 (4886-45179) μg/L, n=20) compared with patients with normal admission creatinine (creatinine <120μmol/L: 7930 (1742-30769) μg/L, n=51, p=0.127, Mann-Whitney U test) on admission to the SLTU. Analysis of admission ferritin levels in both cohorts of paracetamol patients to predict death or requirement for OLT showed an area under the receiver operator characteristic (AUROC) of 0.722 (95% confidence intervals 0.614-0.831, p=0.001), **figure 5.2**, compared with an AUROC for serum ALT of 0.514 (95% CI 0.383-0.645, p=0.829). In contrast with the prognostic significance of ferritin in paracetamol-induced acute liver injury, there were no significant differences noted

in the non-paracetamol acute liver injury patients in those who survived spontaneously or underwent OLT or died (data not shown).

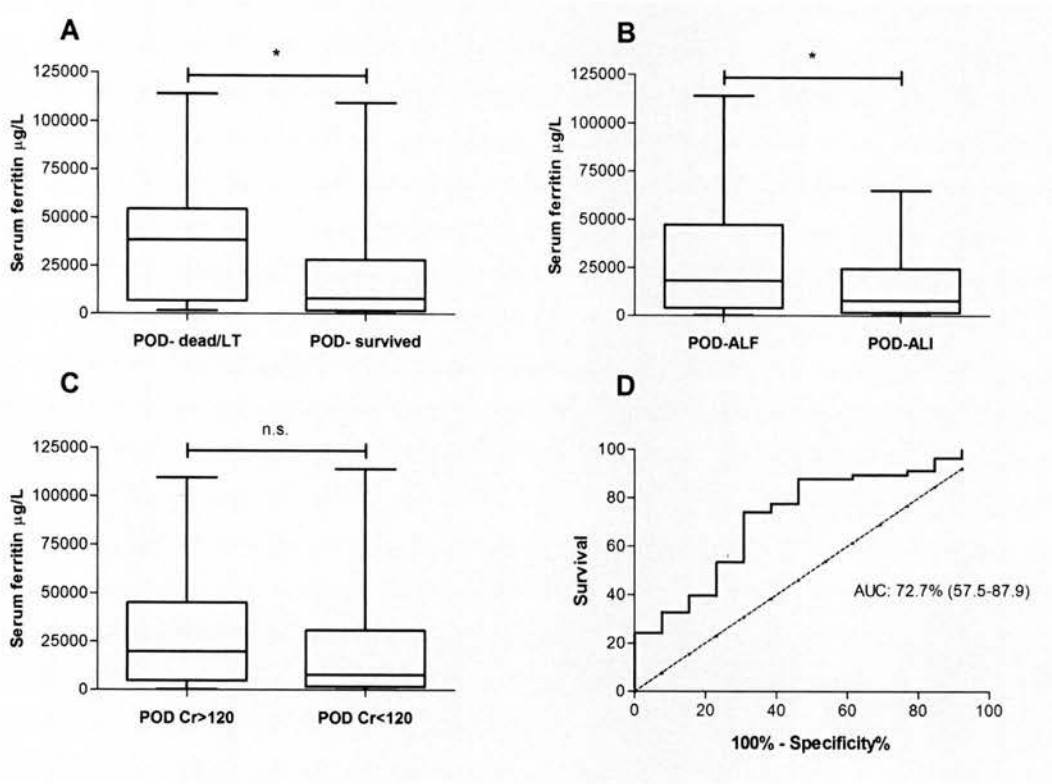

Figure 5.2 Elevated admission serum ferritin is associated with deleterious outcomes following paracetamol overdose.

Retrospective analysis of admission serum ferritin amongst 71 paracetamol-induced acute liver injury patients. (A) Admission ferritin levels amongst patients who died or required liver transplantation (LT) compared with spontaneous survivors. (B) Admission ferritin levels amongst patients who developed HE (ALF) compared with patients without HE. (C) Admission ferritin levels in patients with and without abnormal admission renal function (serum creatinine >120 mmol/L). (D) Receiver operator characteristic curve of admission serum ferritin. Box and whiskers plots represent median, quartiles, and extreme data values. * $p<0.05$, Mann- Whitney U test; n.s. not significant ALF, acute liver failure; ALI, acute liver injury; LT, liver transplantation; AUC, area under the curve

5.4.6 Prospective study confirms significant hyperferritinaemia in paracetamol-induced acute liver injury.

In view of the potential selection bias in analysis of retrospective data serum ferritin was measured in 47 sequential cases of POD acute liver injury admitted to the SLTU. The demographics and admission laboratory parameters and clinical outcomes of this cohort are displayed in **table 5.3**. A total of 27 (57.4%) of these patients developed HE (grade III/IV in 19 (40.4%)), and 13 patients subsequently died or required emergency OLT. Serum ferritin was elevated (i.e. serum ferritin >300 μg/L) in 40 patients (85.1%). Very high serum ferritin was also frequently observed in this cohort (22/47, 46.8% patients had ferritin > 10 000μg/L, 2/47, 4.2% patients had ferritin > 100 000μg/L). Admission serum ferritin was again significantly higher in patients who died or were transplanted (21151, (9443-40130) μg/L; n=13) compared with spontaneous survivors (4237, (776-18020) μg/L; n=34; p=0.013, Mann-Whitney U test). Similarly, admission ferritin levels were significantly higher in paracetamol-induced ALF patients (15465 (6626-33245) μg/L, n=27) compared with paracetamol acute liver injury patients (998.5 (424.3-12040) μg/L, n=20, p=0.005, Mann-Whitney U test). Serial analysis of serum ferritin levels amongst paracetamol-induced ALF patients who died or required liver transplantation (n=10) and spontaneous survivors (n= 14) did not reveal any significant differences between the two groups during the first 3 days of admission (**figure 5.3**).

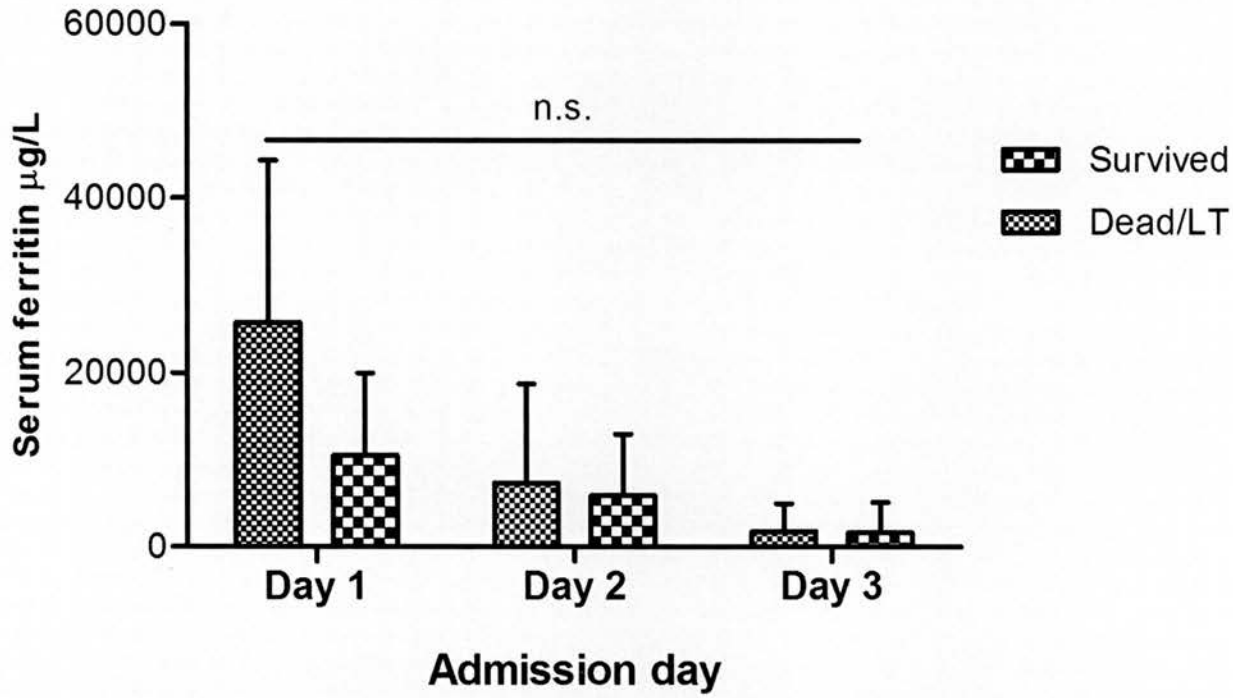

Figure 5.3 Serial analysis of serum ferritin levels amongst paracetamol-induced ALF patients who died or required liver transplantation (n=10) and spontaneous survivors (n= 14).

Data are presented as median +/- interquartile range. There were no significant differences between the two groups over time (2-way analysis of variance)

Analysis of explanted liver tissue (n=5) again did not show significant iron accumulation. Serum ferritin was significantly correlated with both PT (r=0.497, $p<0.001$) and ALT levels (r=0.392, $p=0.006$). In this prospective cohort admission ferritin was also significantly correlated with admission and maximal organ dysfunction scores (**figure 5.4**). Patients that developed ≥2 SIRS components during admission also had elevated admission ferritin (SIRS 14980 (7390-36944) μg/L, n= 24; no SIRS, 1047 (518-18020) μg/L, n=23, p=0.008, Mann-Whitney U test).(Anonymous 1992) There was no correlation with the acute phase response as determined by circulating C-reactive protein, although ferritin was significantly correlated with both proinflammatory (IL-6 and IL-8) and anti-inflammatory (IL-10) cytokine release following POD (**table 5.4**).

Variable		Died (n=8) or transplanted (n=5)	Spontaneous survivors (n=34)
Age		45 (41-48) *	34 (26-41)
Male:Female		4:9	14:20
APACHE II score §		18 (12-27) *	6 (3-10)
SOFA score		12 (8-17) *	3 (2-6)
Admission laboratory parameters	Ferritin (μg/L) (NR = 20-300μg/L)	21151 (9443-40130) *	4237 (776-18020)
	Bilirubin (μmol/L) (NR 3-17 μmol/L)	97 (80-107)	68 (28-121)
	Leucocyte count (x10^9/L) (NR = 4-11 x10^9/L)	7.2 (4.4-9.3)	8.2 (6.3-11.4)
	Platelets (x10^9/L) (NR 150-400 x10^9/L)	74 (49-100) *	163 (91-193)
	Haemoglobin (g/L) (NR 110-180 g/L)	90 (83-131) *	128 (119-137)
	ALT (IU/L) (NR 7-50 IU/L)	6226 (3334-7673)	5198 (3597-7994)
	Creatinine (mmol/L) (NR 60-110 mmol/L)	224 (142-269) *	72 (55-192)
	Prothrombin time (seconds) (NR 12-14 seconds)	76 (37-119) *	41 (30-73)
	IL-6 (pg/mL)	2207 (612-5000) *	162 (48-270)
	IL-8 (pg/mL)	2082 (1011-2785) *	262 (70-700)
	IL-10 (pg/mL)	573 (186-1515) *	51 (18-115)

Table 5.3 Demographic and admission laboratory data for prospectively enrolled paracetamol acute liver injury patients.

*= $p<0.05$, Mann-Whitney U test; NR, normal rangeAPACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sepsis-related Organ Failure Assessment; Data are presented as median +/- IQR unless otherwise indicated

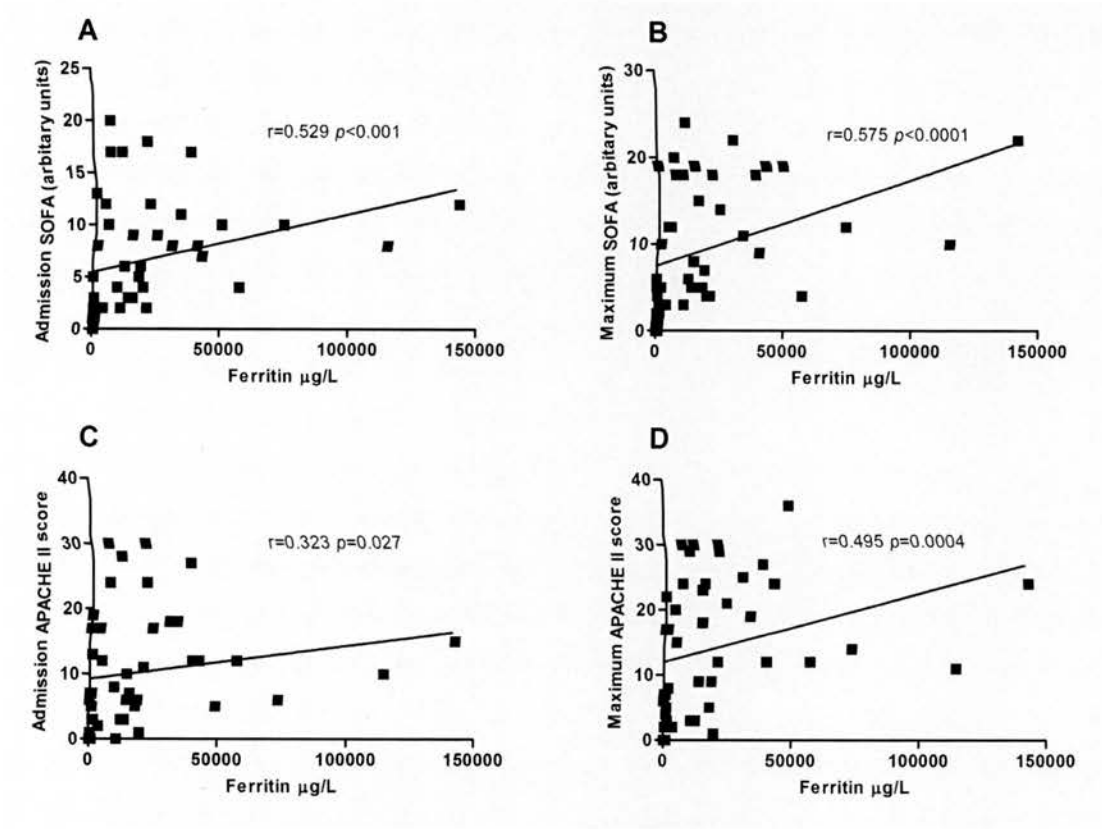

Figure 5.3 Hyperferritinaemia following paracetamol overdose is associated with organ dysfunction

Admission (A) and maximum (B) SOFA score vs. admission ferritin. Admission (C) and maximum (D) APACHE II scores vs. admission ferritin. Correlations between variables were analyzed using Spearman's rank correlation.

Admission variable	Correlation coefficient (Spearman)	*p* value
ALT (IU/L)	0.392	0.006
CRP	0.095	0.525
SOFA score	0.529	<0.001
APACHE II score	0.323	0.027
IL-6 (pg/mL)	0.442	0.006
IL-8 (pg/mL)	0.502	0.001
IL-10 (pg/mL)	0.349	0.030

Table 5.4. Correlation between serum ferritin and clinical and biochemical markers of injury and inflammation.

CRP, C-reactive protein; SOFA, Sequential Organ Failure Assessment; APACHE, Acute Physiology and Chronic Health Evaluation; IL, interleukin

Correlations between variables were analysed using Spearman's rank correlation.

5.4.7 Elevated serum ferritin in acute liver injury is associated with significant levels of circulating H-ferritin

Under normal physiological conditions, the majority of circulating serum ferritin is composed of L-ferritin. Previous studies have suggested an immunomodulatory role for H-ferritin, but to date, increased circulating H-ferritin has not been demonstrated in liver disease.(Li and others 2010) Immunoblotting of serum from acute liver injury patients with elevated circulating ferritin revealed circulating L-ferritin, but also significant amounts of H-ferritin (**figure 5.5**). In contrast, no circulating H-ferritin was observed in healthy controls even after prolonged (15 minutes) exposure (**figure 5.5**).

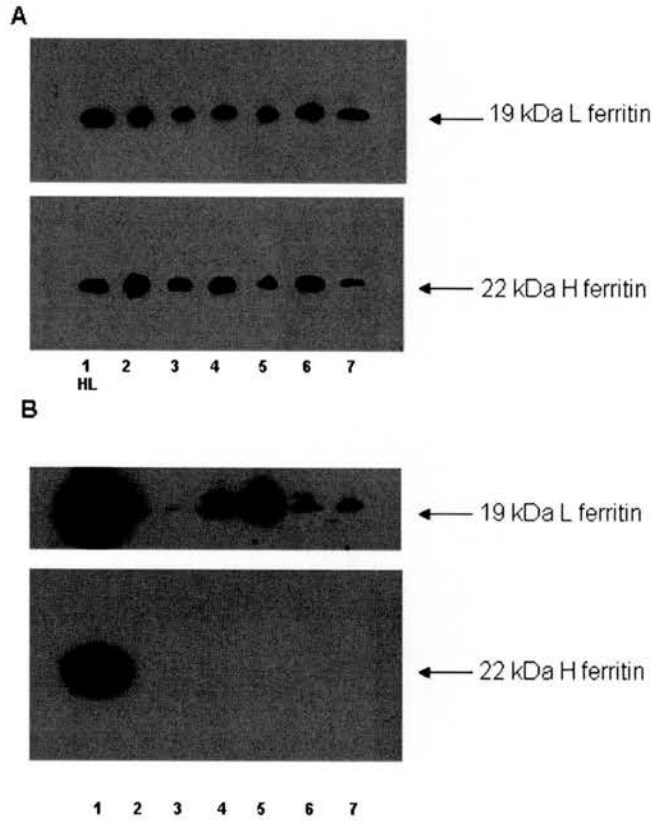

Figure 5.4 The H- and L-ferritin isoforms circulate in patients with paracetamol-induced ALF (continued overleaf)

Figure 5.4 (continued) (A) Representative western blot of L- (19 kDa) and H- (22 kDa) ferritin isoforms in 6 paracetamol-ALF patients (lanes 2-7). All experiments were repeated in triplicate.

Lane	Admission serum ferritin (μg/L)	Outcome
1	Human liver lysate	-
2	49192	Died
3	15465	Survived
4	21130	Died
5	14417	Survived
6	6706	Died
7	10721	Survived

(B) The L- but not the H-ferritin isoform is present in the serum of healthy controls.

Representative prolonged exposure (15 minutes) western blot of 6 healthy controls (lanes 2-7) with no history of iron overload or iron deficiency. Lane 1 = human liver lysate.

5.5 Discussion

This chapter reports extreme elevations of serum ferritin on admission in patients with paracetamol-induced acute liver injury; more than 50% of patients had admission ferritins greater than 10 000 μg/L, while some patients had ferritin concentrations greater than 100 000 μg/L. The elevated serum ferritins are some of the highest values ever reported in the literature. Elevated serum ferritin was associated with increased serum iron concentrations, elevated transferrin saturation, and reduced transferrin concentrations, particularly amongst paracetamol patients. Increased serum ferritin was associated with a more complicated clinical course and worse clinical outcomes in patients with paracetamol-induced acute liver injury, but not those with non-paracetamol causes, suggesting a potential role for serum ferritin as an early biomarker following POD. Elevated serum ferritin was correlated with the degree of liver failure, as determined by PT, and with the presence of systemic inflammation; suggesting that several parallel pathophysiological processes are involved in the massive elevation of serum ferritin reported. In addition, for the first time, circulating levels of the immunomodulatory H-ferritin are demonstrated in patients with paracetamol-induced ALF, suggesting that ferritin may also play a pathophysiological role in the systemic inflammation and multiorgan failure associated with paracetamol overdose.

This is one of the first studies to examine the significance of elevated circulating ferritin in human acute liver injury. (Eastham, Bell, Douglas 1976) The significance of serum ferritin has been explored in both retrospective and prospective cohorts of acute liver injury patients. Additional strengths of this study include the single centre nature of the study and the correlation of ferritin with other pro- and anti-inflammatory cytokines. However, this is a small study and the results require confirmation in other, larger, prospectively collected populations of acute liver injury patients. An important caveat to these retrospective data is potential selection bias since many paracetamol cases in the SLTU database did not have serum ferritin measured. This may reflect diagnostic confusion in some atypical paracetamol cases, or may simply reflect the increased use of electronically ordered 'liver screens' (which include serum ferritin) by admitting clinicians during more recent years. Comparison was therefore made between paracetamol cases where ferritin had been

measured with the remaining paracetamol cases, and no demographic differences between the groups was noted, except a significantly lower proportion of single intentional paracetamol overdose cases in the ferritin measurement group (**table 5.2**). Any potential selection bias was reduced by additionally measuring serum ferritin prospectively in 47 cases of paracetamol induced liver injury sequentially admitted to the SLTU. Another caveat to these data is the small number and heterogeneous nature of the non-paracetamol cohort. This reflects the patient population referred to the SLTU, in which less than 30% of cases are secondary to non-paracetamol causes.

Serum ferritin is elevated in a wide number of acute and chronic inflammatory conditions, as well as non-specifically during critical illness.(Evensen, Swaak, Nossent 2007; Knovich and others 2009; Rambod, Kovesdy, Kalantar-Zadeh 2008; Recalcati and others 2008; You and Wang 2005) It could be argued that the elevated ferritins seen in this study are simply an epiphenomenon reflecting the degree of underlying hepatic necrosis. Several factors argue against this: firstly, the degree of elevation of serum ferritin is greatly beyond that normally seen in patients with acute inflammatory conditions such as acute respiratory distress syndrome, trauma or sepsis.(Garcia and others 2007; Sharkey and others 1999) Secondly, the pattern of increased serum ferritin, transferrin saturations and iron and reduced transferrin concentration is out of keeping with the anaemia of critical illness, making this explanation unlikely.(Walsh and Saleh 2006) Thirdly, although serum ferritin correlated weakly with ALT levels (in the prospective cohort only), ferritin appears more to reflect underlying systemic immune activation (as judged by significant correlations with IL-6, IL-8, and IL-10) and degree of organ injury than simply hepatocyte necrosis. This is reflected in the significant AUROC value for ferritin compared with the non-discriminatory AUROC for ALT. Elevated ferritin is also common in patients with chronic renal failure and relates to clinical outcomes.(Rambod, Kovesdy, Kalantar-Zadeh 2008) The elevated serum ferritin is thought to relate to chronic inflammation in this condition, but could also reflect impaired urinary ferritin excretion.(Chow and others 1995) Elevated serum ferritin has also been reported in patients with acute renal failure.(Gulcelik and Kayatas 2002) However, the levels of ferritin reported in either acute or chronic renal failure are relatively mild compared with the concentrations observed in this study, suggesting that additional mechanisms underlie the hyperferritinaemia of acute liver injury. The increased ferritin levels could also reflect

unrecognized haemochromatosis, and HFE gene testing was not performed in our cohorts to exclude this possibility. However, none of the liver explants showed significant iron overload, and the clinical presentation and degree of hyperferritinaemia in these patients make unrecognised haemochromatosis very unlikely. Extreme elevations of serum ferritin also occur in Still's disease and haemophagocytic lymphohisticytosis, a heterogeneous disorder characterised by hyperferritinaemia, hypertriglyceridaemia, and multiorgan failure.(Emmenegger and others 2005; Evensen, Swaak, Nossent 2007) Interestingly, the latter rare condition may be induced by hepatitis virus infection and has been reported to occur in children with ALF,(Natsheh and others 2001; Shneider, Selsky, Komp 1992) so clinicians dealing with acute liver injury patients should remain vigilant for the possibility of haemophagocytic lymphohisticytosis, particularly given the clinical similarities between these conditions and severe sepsis, SIRS, and ALF.(Castillo and Carcillo 2009)

Despite the widespread use of ferritin as a marker of iron stores, surprisingly little is known regarding its secretion, regulation, or cellular receptors.(Recalcati and others 2008) It is recognised that the transcription of H- and L-ferritin subunits are selectively regulated in response to inflammation, with H-ferritin mRNA in particular being induced by tumour necrosis factor-α and IL-1α in both mesenchymal and monocytic cells.(Fahmy and Young 1993) Hepatocytes,(Ghosh, Hevi, Chuck 2004) macrophages (Wesselius, Nelson, Skikne 1994) and Kupffer cells (Ono and Seno 1991) are all known to secrete ferritin, suggesting that in addition to passive release from damaged hepatocytes, the elevated serum ferritin observed in acute liver injury may also arise from active synthesis and release from undamaged hepatocytes and the reticuloendothelial system. This study is the first to show that H-ferritin is present in circulating form following acute liver injury. Interestingly, treatment of human macrophages with IL-4 to induce an alternative (M2) macrophage phenotype results in enhanced iron release,(Recalcati and others 2010) a finding which has particular resonance for paracetamol-induced liver injury, where the pathophysiological role of macrophages is increasingly recognised,(Possamai and others 2010) and where alternatively activated macrophages are thought to infiltrate the liver following paracetamol injury.(Holt, Cheng, Ju 2008)

Given the association between elevated ferritin and deleterious outcomes in paracetamol-induced ALF it is tempting to speculate on the potential role for elevated ferritin in the

pathogenesis of this condition. H-ferritin receptors have been identified on hepatocytes, kidney cells, and B- and T-lymphocytes, and this receptor in humans has recently been identified as transferrin receptor-1.(Li and others 2010) Exogenous ferritin is avidly taken up by hepatocytes, a process which may stimulate apoptosis through the FasL/Fas pathway.(Bresgen and others 2007) Recently, it was shown that at high concentrations ferritin can also trigger necrotic hepatocyte death through increased lysosomal membrane permeability,(Bresgen and others 2010) suggesting that ferritin might indirectly modulate the type of cell death seen following paracetamol injury.(Gujral and others 2002) Ferritin can also inhibit lymphocyte proliferation, downregulate chemokine signalling and the delayed hypersensitivity response, and stimulate production of the immune regulatory cytokine IL-10.(Wang and others 2010) Future studies should therefore explore whether hyperferritinaemia contributes to the increased risk of infection following acute liver injury.(Antoniades and others 2008; Berry and others 2010)

Increased serum ferritin is common in patients with chronic liver disease and has been linked to mortality on the liver transplant waiting list.(Walker and others 2010) Elevated serum ferritin is also common in patients with alcoholic hepatitis,(Bell and others 1994) hepatitis C infection,(Ferrara and others 2009) and non-alcoholic fatty liver disease, and future studies should therefore explore whether H-ferritin levels are also increased in these conditions.(Licata and others 2009) However, the degree of hyperferritinaemia seen in paracetamol-induced ALF patients suggests that much greater derangements of the innate immune system occur in this condition. Significantly higher serum ferritin levels were found in paracetamol patients who died or were transplanted compared with spontaneous survivors. In contrast, analysis of the retrospective cohort revealed that admission serum ferritin was not predictive of outcome in the non-paracetamol cohort. This may be due to the rapid clinical evolution of the paracetamol-induced acute liver injury patients, with clinical outcomes occurring much earlier in this group of patients compared with patients with non-paracetamol induced acute liver injury. These data suggest that serum ferritin may have potential as an early biomarker following paracetamol injury, particularly given its widespread availability in clinical practice, but this possibility should be explored in larger cohorts of paracetamol patients.

In conclusion, this chapter demonstrates extreme hyperferritinaemia following acute liver injury. Hyperferritinaemia was associated with a more complicated clinical course and worse outcomes in patients with paracetamol-induced acute liver injury, and correlated with the degree of liver failure and systemic inflammation. In addition, increased circulating levels of the immunomodulatory H-ferritin are demonstrated in patients with paracetamol-induced ALF. Future studies should explore the pathophysiological role of ferritin in acute liver injury, and examine the utility of ferritin as a biomarker following paracetamol-induced liver injury.

Chapter 6. Circulating levels of the long pentraxin PTX3, but not hepatocyte derived C-reactive protein, correlate with severity following human acute liver injury

6.1 Introduction

6.2 Materials and methods

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6.3.5 Correlation of PTX3 and CRP with other markers of disease severity and systemic inflammation in paracetamol-induced acute liver injury

6.3.6 Time course of PTX3 and impact of liver transplantation

6.4 Discussion

Chapter 6. Circulating levels of the long pentraxin PTX3, but not hepatocyte derived C-reactive protein, correlate with severity following human acute liver injury

6.1 Introduction

The previous chapters, and other published studies, have highlighted the association between development of the SIRS following both paracetamol and non-paracetamol induced acute liver injury and deleterious clinical outcomes.(Leithead and others 2009; Rolando and others 2000b; Vaquero and others 2003) As described previously, defective functioning of the cellular components of the innate immune system, in particular monocyte-macrophages,(Holt, Cheng, Ju 2008) dendritic cells,(Chen and others 2009) and neutrophils,(Liu and others 2006) have all been implicated in the pathophysiology of ALF, but to date, few studies have focused upon the humoral arm of the innate immune system. The humoral innate immune system consists of several soluble factors, such as the pentraxins, ficolins, and collectins. The pentraxins are a superfamily of highly evolutionarily conserved soluble pattern recognition receptors (PRRs) with a wide range of functions in inflammatory diseases.(Bottazzi and others 2010) The best characterised are the short pentraxins, which include C-reactive protein (CRP) in humans and serum amyloid P component (SAP) in mice, which are produced predominantly by hepatocytes in response to circulating proinflammatory mediators including interleukin (IL)-6. Pentraxin 3 (PTX3) is the prototypical long pentraxin which is rapidly synthesised and released in response to both cytokines, including IL-1, tumour necrosis factor-α and IL-10, and toll-like receptor engagement.(Doni and others 2006; Mantovani, Garlanda, Bottazzi 2003) Diverse effector functions of PTX3 include complement binding, opsonisation of bacteria, and the handling of apoptotic cells.(Manfredi and others 2008) Elevated levels of PTX3 have been demonstrated in a wide variety of proinflammatory conditions including sepsis and acute myocardial infarction, with PTX3 levels strongly correlating with disease severity in these conditions.(Fazzini and others 2001; Latini R. and the Lipid Assessment Trial Italian Network (LATIN) Investigators 2004; Luchetti and others 2000; Muller and others 2001)

Previous studies have suggested that serum CRP levels are relatively suppressed in ALF patients with concomitant septic complications and that the relatively reduced levels of CRP

in these patients contributed to the increased risk of infection.(Silvestre, Coelho, Póvoa 2010) PTX3 is produced predominantly by endothelial cells and monocytes,(Alles and others 1994) whereas CRP is produced almost exclusively by hepatocytes.(Steel and Whitehead 1994) In ALF, especially when caused by paracetamol, the former cell types are activated and induced, in contrast with hepatocytes which are significantly injured and necrotic. It was therefore hypothesised that circulating PTX3 levels would be increased in patients with acute liver injury, elevated in those with worse outcomes, and reduced in patients with infective complications.

6.2 **Materials and methods**

6.2.1 Patients, Study Design, and Definitions

The study was prospectively approved by the Scotland 'A' Research Ethics Committee. A total of 60 consecutive adult patients (24 male, 36 female) admitted to the Royal Infirmary of Edinburgh with ALF or acute liver injury between December 2008 and March 2010 were entered into the study. Informed consent or assent was obtained from all patients or their nominated next of kin respectively prior to study inclusion. Acute liver injury and ALF were defined as in previous chapters. For those patients admitted with presumed paracetamol hepatotoxicity, admission to the study required the concomitant presence of a serum alanine aminotransferase (ALT) >1000 IU/L and any degree of coagulopathy. Aetiologies of liver injury were defined as previously described. Admission laboratory and clinical parameters were prospectively recorded in a dedicated database as previously described.

Patients were managed according to a standardised clinical protocol as described in previous chapters. Healthy and pathological control groups consisted of 13 healthy controls (HC) and 10 clinically stable patients with chronic liver disease (CLD) attending a portal hypertension outpatient clinic.

6.2.2 Blood sampling

Peripheral serum and plasma samples were collected daily in serum gel and potassium-ethylenediaminetetraacetic acid containing plastic tubes (Sarstedt, Leicester, UK) and centrifuged at 1000g for 15 minutes at 4ºC within one hour following collection. Plasma and serum were immediately aliquoted and stored in polypropylene tubes at -80ºC until analysis. In patients undergoing emergency OLT, peripheral blood samples were obtained within 24 hours following transplantation.

6.2.3 Routine variables

Standard haematological, biochemical, and coagulation parameters were obtained on a daily basis during admission.

6.2.4 PTX3 Enzyme-Linked Immunosorbent Assay (ELISA)

Admission plasma PTX3 levels were measured in all patients using a commercial quantitative sandwich ELISA (R&D Systems Inc, Abingdon, UK) according to the manufacturer's instructions. PTX3 levels were additionally measured on day 3-4 of admission in 14 paracetamol-induced ALF patients (see **section 6.3.6**). A 96-well streptavidin-coated plate was incubated with a PTX3 biotinylated monoclonal antibody and incubated for 60 minutes on a microplate shaker. Following washing of the plate, pretreated ethylenediaminetetraacetic acid plasma samples were added to the plate in duplicate. Following incubation at room temperature for 2 hours and washing away of any unbound substances, an enzyme-linked conjugate was added to the wells and incubated for a further 2 hours. Following a wash to remove any unbound conjugate, a substrate solution was added to the wells and colour development measured at 450 nm using an automatic ELISA reader (Tecan Spectra, Crailsheim, Germany). Results were determined from a standard curve prepared from 7 human PTX3 standards, with a lower threshold of detection of 0.31 ng/mL. All pathological samples were above the detection limit, but a total of 6/13 (46.2%) of HC samples were below the lower threshold of detection. The coefficient of variation was 3.6%.

6.2.5 Highly sensitive C-reactive protein

Admission serum CRP levels were analysed in liver injury patients using a turbimetric method using a Beckman AU2700 system (Beckman Coulter Ltd., High Wycombe, UK). CRP levels were additionally measured on day 3-4 of admission in 14 patients (see **section 6.3.6**). The coefficient of variation of this system was 5% at values <2 mg/L and 2-3% at higher concentrations.

6.2.6 Serum IL-6 and IL-10

Admission cytokine measurements were performed using a cytometric bead array kit and software (BD Biosciences, San Jose, CA.) as described in **chapter 5**.

6.2.7 Statistical Analysis

Data values are presented as median and interquartile range or percentages unless otherwise stated. Statistical analysis was performed using SPSS (SPSS 16.0, Chicago IL, USA) and Graphpad Prism (GraphPad Software Inc., La Jolla, CA). Continuous data were compared using either analysis of variance or the Kruskal-Wallis test if inter-group variances were

unequal, with post-hoc Dunn's testing used to compare selected groups. Categorical data were analysed using Chi-squared tests or Fishers exact test. Correlations between PTX3 levels and other variables were analyzed using Spearman's rank correlation. Stepwise logistic regression was used to determine factors predictive of death or OLT in the acute severe liver injury cohort as a whole, and then separately for the paracetamol patients. Wilcoxon matched pairs test was used to compare repeated measures. Results were considered statistically significant when two-sided $p < 0.05$.

6.3 Results

6.3.1 Patients

Table 6.1 illustrates the baseline demographic and admission laboratory variables of the liver injury study populations. Paracetamol patients were significantly younger than both non-paracetamol and CLD patients. Paracetamol patients had significantly higher ALT and PT values on admission compared with non-paracetamol patients, but had lower serum bilirubin levels and lower leucocyte counts. Of note is the inclusion in the non-paracetamol group of 2 post-transplant patients with acute liver injury (1 primary non-function, 1 small-for-size syndrome); subsequent analysis excluding these patients had no effect upon the reported results. A total of 28/48 (58.3%) of paracetamol and 8/12 (66.7%) non-paracetamol patients developed HE, and therefore ALF, during the study. Of these patients, 18/28 (64.3%) paracetamol and 5/8 (62.5%) non-paracetamol ALF patients met the KCC, with 8 (5 paracetamol, 3 non-paracetamol) patients subsequently undergoing emergency OLT.

	Paracetamol	Non-Paracetamol	CLD	HC
Number of patients	48	12	10	13
Age	37.5 (29.5-45) *†	58.5 (36.5-65.5)	56 (49-59)	34 (30.5-52.5)
Male:Female	19:29 †	5:7	8:2	5:8
Aetiology	Paracetamol	AIH (3); HBV(2); HCV (1); AFLP (1); PNF (1); SFSS(1); Neoplasia (1); DILI (1); EPP (1)	ALD (7); AIH (1); NAFLD (2)	-
APACHE II score §	7 (4-17)	12.5 (4.5-19)	-	-
SOFA score	5 (2-10.5)	8.5 (4-12.5)	-	-
Bilirubin (μmol/L)	83 (33.5-108) †	199 (118-313) *	17 (9-35)	-
Leucocyte count (x10^9/L)	7.8 (4.9-11.3)	11 (5.5-13.6)	6.3 (4.4-8.2)	-
Platelets (x10^9/L)	121.5 (72-186)	73 (37.5-152)	164 (78-225)	-
Haemoglobin (g/L)	125.5 (110-135.5)	107 (83.5-140.5)	148 (126-154)	-
ALT (IU/L)	5329.5 (3465.5-7983.5) *	1218.5 (281.5-2869.5)	31 (29-38)	-
Creatinine (mmol/L)	113.5 (61-240) †	124 (86-204.5)	70 (57-84)	-
PT (seconds)	43.5 (32-76.5) *	20.5 (15.5-39)	1.0 (1.0-1.2)**	
HE on admission (%)	22 (45.8%)	8 (66.6%)	-	-
CVVH (%)	14 (29.2%)	7 (58.3%)	-	-
Mechanical ventilation (%)	18 (37.5%)	7 (58.3%)	-	-

	Paracetamol	Non-Paracetamol	CLD	HC
Inotropic support (%)	16 (33.3%)	5 (41.7%)	-	-
Met KCC	18 (37.5%)	5 (41.7%)	-	-
Transplanted	5 (27.8%)	4 (80%) *	-	-
Spontaneous survivors	34 (70.8%)	5 (41.7%)	-	-

Table 6.1: Baseline demographic and laboratory values in patients with paracetamol and non-paracetamol induced acute liver injury.

* $p<0.05$ paracetamol v. non-paracetamol/CLD; ** $p<0.01$ paracetamol v. non-paracetamol/CLD; † $p<0.001$ paracetamol v CLD; ‡$p<0.01$ paracetamol v CLD; ξ $p<0.05$ Non-paracetamol v CLD § Calculated at 24 hours post-admission

AIH, autoimmune hepatitis; HBV, hepatitis B; HCV, hepatitis C; AFLP, acute fatty liver of pregnancy; PNF, primary non-function; SFSS, small-for-size syndrome; EPP, erythropoietic protoporphyria; NAFLD, non-alcoholic fatty liver disease CLD, chronic liver disease; HC, healthy control.

6.3.2 Circulating long and short pentraxin levels in acute liver injury

Median admission plasma PTX3 levels were significantly higher in the acute liver injury cohort as a whole (median (IQR): 78.2 (14.6-446.8) ng/mL, n=60) compared with both CLD patients (1.55 (1.22-2.23) ng/mL, n=10, $p<0.0001$) and HC (0.33 (0.31-0.52) ng/mL, n=13, $p<0.0001$). Levels of PTX3 were significantly higher in paracetamol patients (148.6 (26.6-579) ng/mL, n=48) compared with non-paracetamol patients (23.7 (9.1-40.0) ng/mL, n=12, $p=0.004$), **figure 6.1**. In contrast with the elevated levels of PTX3 in the paracetamol patients, admission CRP levels were significantly increased in non-paracetamol acute liver injury patients (17.55 (3.93-15.38) mg/L, n=12) compared with paracetamol patients (6.05 (3.93-15.38) mg/L, n=48, $p=0.011$), **figure 6.1**.

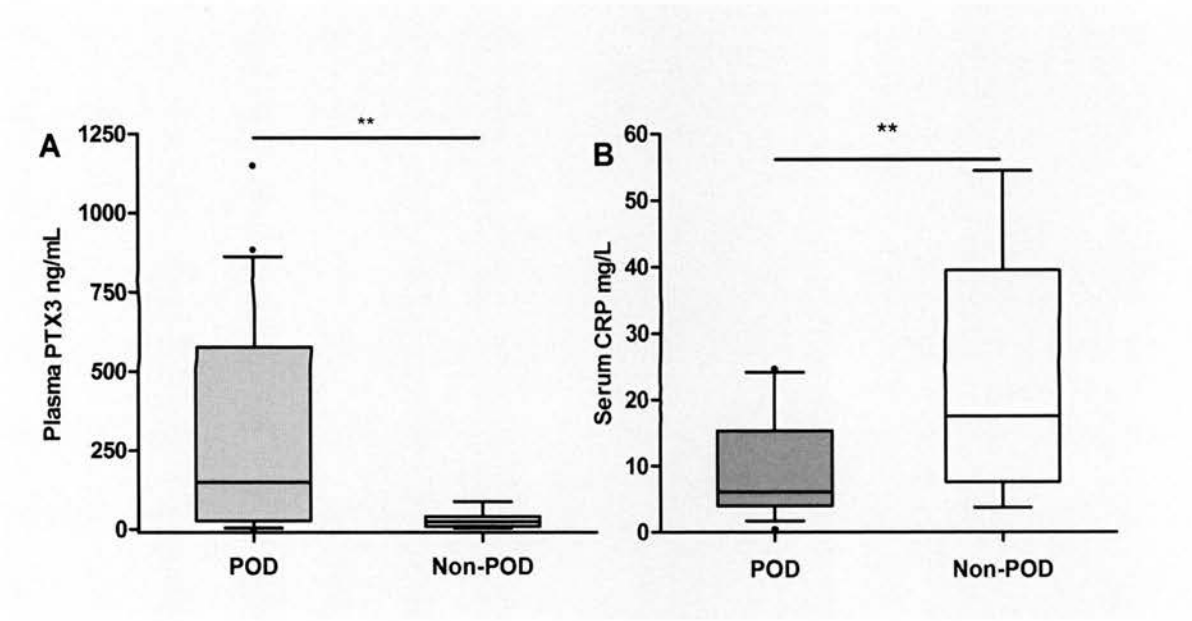

Figure 6.1 PTX3 (A) and CRP (B) levels in paracetamol (n=48) and non-paracetamol (n=12) patients admitted with acute severe liver injury.

Box and whiskers plot represents median, interquartile range, and 5-95% range respectively. POD, paracetamol overdose. ** $p<0.01$

6.3.3 Relation of PTX3 levels to outcome and disease severity in paracetamol patients

Admission PTX3 levels in paracetamol patients who died or required emergency OLT (568.2 (176.4-832.7) ng/mL, n=14) were significantly higher compared with spontaneous survivors (64.3 (10.0-372.3) ng/mL, n=34, p=0.0011). Admission PTX3 levels were also significantly higher in paracetamol patients who developed HE during their illness (median (interquartile range) (329.4 (77.7-738.1 ng/mL, n=28) compared with acute liver injury patients (no HE) (46.1 (6.1-172.4) ng/mL, n=20, p=0.0005). Of those paracetamol patients who developed HE, admission PTX3 levels showed a trend towards higher levels in patients who met the KCC (452.9 (142.9-831.4) ng/mL, n=18) compared with patients not meeting the KCC (155.3 (41.3-599.1), n=10, p=0.0722). The area under the ROC for PTX3 for the outcome of death/ requirement for OLT was 0.80 (95% confidence intervals 0.67- 0.93), **figure 6.2.**

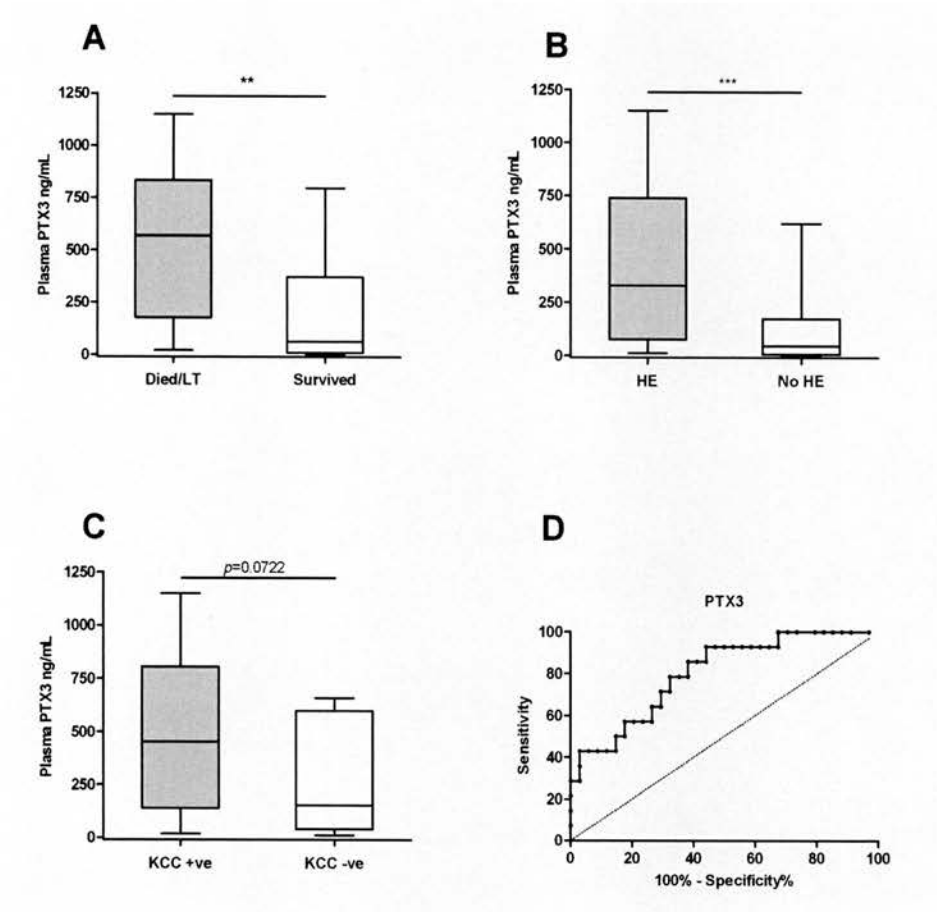

Figure 6.2 Outcomes in paracetamol overdose patients according to admission PTX3 levels. (A) Death or liver transplantation (LT) vs. spontaneous survival. (B) Development of HE during illness. (C) Fulfillment of KCH poor prognostic criteria. (D) Receiver operator characteristic (ROC) of admission PTX3 levels for death/LT or spontaneous survival. *** $p<0.001$

Admission PTX3 levels in paracetamol patients correlated with admission APACHE II (r=0.398, p=0.006) and SOFA (r=0.536, p<0.001) scores. In addition, admission PTX3 levels were more strongly correlated with the maximal SOFA score during admission (r=0.603, p<0.001). (**Figure 6.3**)

6.3.4. Relation of PTX3 levels, systemic inflammation, and infection in paracetamol patients

Admission PTX3 levels were higher in paracetamol patients who had developed the SIRS by the time of admission (306.4 (87.4-772.9) ng/mL, n=21) compared with no systemic inflammatory response (66.1 (7.99-352.3) ng/mL n=27, p=0.018) and with development of the SIRS at any stage during admission (SIRS 306.4 (113.1-772.9) ng/mL, n=25; no SIRS 50.5 (6.7-297.7) ng/mL, n=23, p=<0.001), **figure 6.3**. A total of 17 patients subsequently developed bacteriologically confirmed infection, including staphylococcal bacteraemia (n=5), candidaemia (n=4), *E. coli* (n=4), anaerobic infections (n=2), mycobacterial infection (n=1), and other Gram positive bacteraemias (n=2). Admission PTX3 levels amongst those patients subsequently developing an infection (142.9 (41.6-619.6) ng/mL) were not significantly different to patients without infection (57.9 (10.0-306.4) ng/mL, p=0.518).

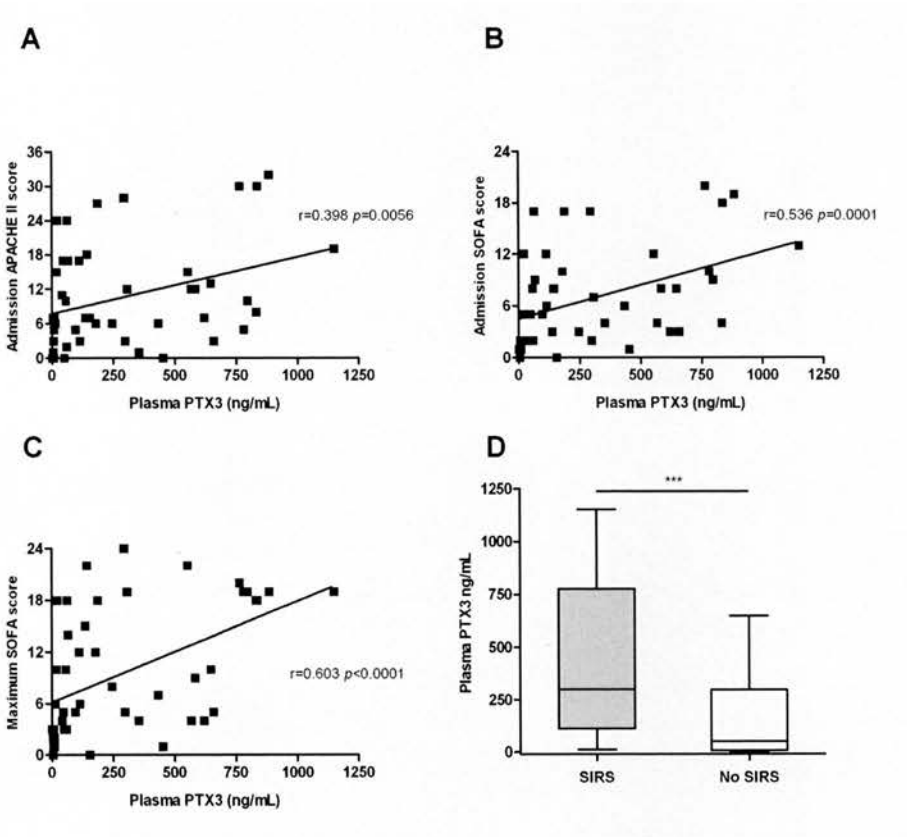

Figure 6.3 Admission PTX3 levels are associated with the development of multiorgan failure in paracetamol patients. Correlations between variables were analysed using Spearman's rank correlation. (A) Admission APACHE II scores vs. admission PTX3. (B) Admission SOFA score vs. admission PTX3. (C) Maximum SOFA score during admission vs. admission PTX3. (D) Development of the SIRS during admission. Box and whiskers plots represent median, quartiles, and extreme data values. *** $p<0.001$

6.3.5. Correlation of PTX3 and CRP with other markers of disease severity and systemic inflammation in paracetamol-induced acute liver injury

The levels of PTX3 and CRP were then correlated with a number of markers of disease severity in both the paracetamol and non-paracetamol cohorts, with the results shown in **table 6.2**. PTX3 showed a strong correlation in both groups of patients with IL-10 and IL-6, as well as the multiorgan failure markers outlined above. In addition, PTX3 correlated with serum ALT, creatinine, and INR. In contrast, CRP levels showed little correlation with indices of disease severity. Multivariate logistic regression was performed to determine whether PTX3 levels were an independent predictor of outcome. As shown in **table 6.3**, only admission SOFA scores independently predicted death or OLT.

Admission parameter	Non-paracetamol patients (n=12)				Paracetamol patients (n=48)			
	PTX3		CRP		PTX3		CRP	
	r	*p*	r	*p*	r	*p*	r	*p*
CRP (mg/L)	**-0.049**	**0.740**	-	-	-0.049	0.740	-	-
IL-6 (pg/mL)	**0.548**	**<0.001**	-0.050	0.765	**0.550**	**<0.001**	0.050	0.765
IL-10 (pg/mL)	0.720	<0.001	**-0.102**	**0.537**	**0.810**	**<0.001**	-0.102	0.537
APACHE II score §	0.422	0.003	**0.198**	**0.177**	**0.398**	**0.006**	0.198	0.177
SOFA score	0.546	<0.001	**0.143**	**0.332**	**0.536**	**<0.001**	0.143	0.332
ALT (IU/L)	0.433	0.002	-0.148	0.314	**0.475**	**0.001**	-0.148	0.314
Creatinine (mmol/L)	0.444	0.002	**0.174**	**0.238**	**0.424**	**0.003**	0.174	0.238
INR	0.615	<0.001	-0.180	0.232	**0.601**	**<0.001**	-0.180	0.232

Table 6.2. Correlation between PTX3 and CRP levels, liver injury, and immune activation in non-paracetamol and paracetamol cohorts.

Correlations between variables were analysed using Spearman's rank correlation.

Variable	All patients (n=60)		Paracetamol overdose (n=48)	
	Multivariate OR (95% CI)	*p* Value	Multivariate OR (95% CI)	*p* Value
Admission PTX3 level	1.001 (0.998-1.004)	0.589	1.002 (1.000-1.006)	0.204
Encephalopathy on admission	1.277 (0.160-10.201)	0.818	1.229 (0.139-10.860)	0.853
Admission ALT	1.000 (1.000-1.000)	0.295	1.000 (1.000-1.000)	0.886
Admission creatinine	0.998 (0.993-1.004)	0.571	1.000 (0.994-1.006)	0.993
Admission PT	1.016 (0.983-1.050)	0.352	1.006 (0.973-1.040)	0.734
Admission SOFA score	1.442 (1.130-1.841)	0.003	1.415 (1.084-1.849)	0.011

Table 6.3 Factors predictive of mortality on stepwise multivariate analysis of admission parameters in all (n=60) and paracetamol only (n=48) patients admitted with acute severe liver injury.

6.3.6. Time course of PTX3 and impact of liver transplantation

Given that admission PTX3 was correlated with subsequent poor outcomes, it was hypothesised that PTX3 levels would remain persistently elevated in patients who died or were transplanted. To test this, PTX3 levels were measured on admission and again at day 3-4 post-admission in 8 paracetamol-induced ALF patients who died or later underwent emergency OLT, and compared with 6 paracetamol-induced ALF patients who spontaneously survived. As shown in **figure 6.4**, PTX3 levels fell significantly in both groups by day 3-4. The impact of impaired liver function on circulating PTX3 and CRP was then explored by measuring circulating levels before and after emergency OLT in 5 paracetamol-induced ALF patients. As shown in **figure 6.4**, post-operative PTX3 levels fell markedly; in contrast there was a rapid increase in CRP levels following OLT.

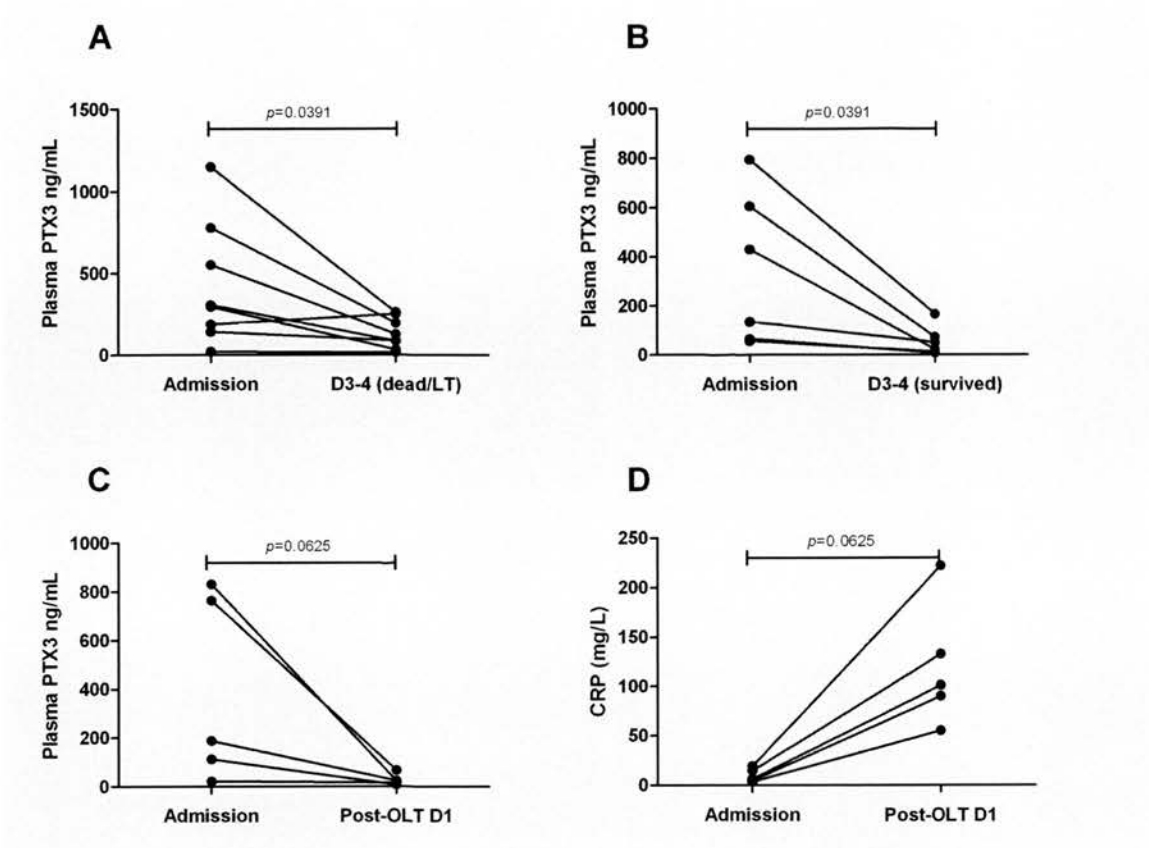

Figure 6.4 Serial levels of pentraxins in paracetamol-ALF patients.

Serial PTX3 levels in paracetamol-ALF patients who (A) died or required OLT (n=8) and (B) spontaneous survivors (n=6). Impact of OLT upon PTX3 (C) and CRP levels (D) in 5 paracetamol patients. Samples were taken within the first 24 hours following hepatic reperfusion.

6.4 Discussion

This observational cohort study demonstrates exceptionally high levels of PTX3 following paracetamol-induced acute liver injury in contrast with non-paracetamol acute liver injury. In the paracetamol cohort PTX3 levels correlated with liver injury, organ failure, and adverse outcomes. PTX3 levels were elevated in patients with the SIRS and correlated with the cytokines IL-6 and IL-10, but were similar in infected and non-infected cohorts. Levels of PTX3 fell rapidly following admission in both survivors and those who died or were transplanted. Removal of the injured liver with transplantation resulted in rapid falls in circulating PTX3. In contrast with PTX3, levels of CRP, a hepatocyte derived short pentraxin involved in the acute phase response, were most elevated in patients with non-paracetamol acute liver injury, bore little relationship to eventual outcome, and significantly increased following liver transplantation. These data demonstrate that the humoral arm of the innate immune system is strongly and selectively activated following acute liver injury, and may be implicated in the development of downstream multiorgan failure. These data also support the hypothesis that it is the degree of immune system activation, rather than the degree of hepatocyte injury *per se*, that is the main determinant of outcome following paracetamol-induced acute liver injury.

The strengths of this study include the simultaneous measurement of two pentraxins in the same cohort of patients with a wide range of severity of liver injury, and the correlation with other pro- and anti-inflammatory markers. Additionally, changes in circulating PTX3 over time have been examined in both spontaneous survivors and patients who died, as well as assessing the impact on PTX3 levels of removal and replacement of the injured liver with functional liver mass by OLT. A further strength of this study is its single centre nature, where all the patients were managed according to a predefined clinical protocol which remained unchanged throughout the duration of the study.

However, this is a small study and the results will require confirmation in other, larger, populations of acute liver injury patients. Due to the difficulties with obtaining liver biopsy

specimens from patients with acute liver injury, the cellular source of PTX3 could not be histologically confirmed, but, given the soluble nature and cellular sources of PTX3, the benefits of hepatic tissue examination in this scenario may be limited. Furthermore, the highest levels of PTX3 were observed on admission and had considerably reduced by the time patients came to transplantation. It is also recognised that not all of the patients included in this study developed HE, and therefore ALF. Another caveat to these data is the small number and heterogeneous nature of the non-paracetamol cohort which reflects the patient population referred to the SLTU, where less than 30% of cases are secondary to non-paracetamol causes. There was a clear difference observed in the circulating PTX3 levels in paracetamol and non-paracetamol cases, but future studies should determine whether measuring PTX3 is of benefit in excluding paracetamol as a cause in ALF cases of aetiological uncertainty.

PTX3 is the prototypical long pentraxin which is present at very low levels (<2 ng/mL) in normal humans, as was observed in our normal controls. Little is currently known regarding the natural half-life of PTX3 in humans and the effects of organ failure on the clearance of PTX3.(Zhang, Danas, Preisner 2010) Whilst the short pentraxins human CRP and murine SAP are produced in the liver in response to IL-6, PTX3 originates predominantly from extrahepatic sources, in particular vascular endothelium, fibroblasts, macrophages, and myeloid dendritic cells.(Mantovani, Garlanda, Bottazzi 2003) Recently, PTX3 was demonstrated to exist in a ready-made form in neutrophilic granules (Garlanda and others 2002) and to localise in neutrophil extracellular traps (NETs),(Brinkmann and others 2004) suggesting that neutrophils, one of the first cellular types attracted to the liver following acute liver injury,(Jaeschke and Hasegawa 2006) may act as a reservoir for PTX3 prior to its biosynthesis by other cell types.

In contrast with the divergence between human and murine species regarding the short pentraxins, PTX3 is highly conserved in humans and mice. Existing experimental data suggest that PTX3 plays an important role in the response to infection or tissue injury. Transgenic mice overexpressing PTX3 display enhanced resistance to caecal ligation and puncture,(Dias and others 2001) whilst *ptx-3* deficient mice have increased susceptibility to myocardial damage following ischaemia-reperfusion injury, a phenotype which can be

reversed by exogenous PTX3.(Salio and others 2008) These data show that PTX3 release is massively upregulated following paracetamol injury and the correlation with organ failure and outcome suggest a potential pathogenic role for PTX3 in the development of multiorgan failure in paracetamol-induced ALF. PTX3 may play an important role in the modulation of local tissue coagulation following infection or inflammation. Levels of PTX3 are increased in patients with small vessel vasculitides,(Fazzini and others 2001) and PTX3 is known to modulate the complement system, predominantly through binding of C1q, leading to activation of the classical complement cascade.(Nauta and others 2003) Additionally, PTX3 upregulates tissue factor (TF) expression in both endothelial cells and activated monocytes,(Napoleone and others 2002; Napoleone and others 2004) suggesting that PTX3 may amplify local clotting cascades and cause a shift towards a procoagulant state. The role of TF in the mediation of endothelial damage following paracetamol injury is gaining increasing recognition,(Kerr and others 2003) with the suggestion that TF is rapidly upregulated following damage to sinusoidal endothelial cells, leading to disruption of hepatic blood flow and development of localized hypoxia.(Ganey and others 2007b) The upregulation of PTX3 in this study is intriguing since it occurred rapidly following paracetamol injury, and frequently before the development of the severe HE, renal impairment, and coagulopathy required to fulfil the KCC.(O'Grady and others 1989) Furthermore, since PTX3 levels correlated more strongly with maximal, rather than admission, SOFA scores, PTX3 may also have a future role as an early biomarker of organ failure in this condition. Future studies should explore whether the rapid falls in PTX3 levels seen in both survivors and non-survivors reflects exhaustion of neutrophil production or alternatively relates to monocyte-macrophage migration into the hepatic milieu.

The pentraxin family plays an important role in the handling of apoptotic cells and the removal of cellular debris in order to prevent autoimmunity. Pentraxins efficiently bind dying cells,(Familian and others 2001; Gershov and others 2000) and PTX3 was recently shown to bind to the plasma membrane of maturing dendritic cells, enhancing the removal of late apoptotic debris and minimising autoimmunity by reducing cross-presentation to T cells.(Baruah and others 2006) Importantly, PTX3 did not prevent the presentation of exogenous microbial ligands to T cells, demonstrating the flexibility of PTX3 when exposed to external danger signals. These results have bearing upon paracetamol-induced

hepatotoxicity, in which conflicting *in vitro* and animal data have emerged to suggest a potential role for apoptotic cell death in the propagation of liver injury.(El-Hassan and others 2003; Kass and others 2003; Kon and others 2004; Ray and others 1996; Zhang and others 2000) Histological examination of explanted and post-mortem liver sections from patients following paracetamol-induced ALF have demonstrated significant apoptotic indices.(McGregor and others 2003) Interestingly, in one cohort study, autoantibodies were absent in cases of paracetamol-induced ALF, compared with 43% of non-paracetamol cases,(Bernal and others 2007b) and it may be that the extremely elevated PTX3 levels seen following paracetamol injury may help to minimise subsequent autoimmunity.

Another important role for PTX3 is in defence against pathogens, particularly but not exclusively as an opsonin. As described above, PTX3 exists readymade in neutrophils,(Jaillon and others 2007) and plays a non-redundant role in the response to certain viral, bacterial, and fungal infections.(Garlanda and others 2002). PTX3 over-expressing mice are also more resistant to LPS toxicity and caecal ligation and puncture. Bacteriologically proven infection is recorded in up to 80% of ALF patients and fungal infection (predominantly candidiasis) occurs in 32%,(Rolando and others 1990; Rolando and others 1991) and it is generally accepted that the immune response to infection is impaired following acute liver injury. Although there were no significant differences in admission PTX3 levels between patients developing subsequent infections and those who did not, the rapid falls in PTX3 levels seen between day 1 and day 3-4 of admission in paracetamol patients are intriguing, since exhaustion of PTX3 production might contribute to the high frequency of downstream invasive bacterial or fungal infections in ALF.(Antoniades and others 2008)

These data raise questions as to why CRP levels, a short pentraxin with diverse proinflammatory roles including complement activation, upregulation of endothelial adhesion molecules, and inhibition of nitric oxide synthase, should show only modest increases despite intense hepatocyte injury. CRP is produced almost exclusively by hepatocytes, particularly in response to IL-6, yet we found a disassociation between IL-6

levels and CRP. Several other studies in acute liver injury patients have also noted a discrepancy between CRP levels and the degree of systemic inflammation. This suggests that in the presence of intense liver cell necrosis, hepatic synthesis of CRP is impaired, a hypothesis that is supported by our observations following OLT. Izumi *et* al noted relatively suppressed admission levels of CRP in 50 ALF patients, and suggested that the acute phase response following acute liver injury is restricted due to severe hepatic synthetic failure.(Izumi and others 1994) Izumi *et al* also failed to demonstrate a correlation on admission between CRP and IL-6 levels in paracetamol patients, but did find a correlation in patients with viral hepatitis, which suggests that the hepatic response to IL-6 may be particularly impaired following paracetamol injury. Low postoperative CRP levels have been shown to independently predict poor outcomes following liver resections, and are inversely correlated with the extent of liver resection.(Rahman and others 2008) Recently, a small case series of 7 patients with concomitant fulminant hepatic failure and sepsis reinforced the notion that CRP levels are relatively suppressed following massive liver injury.(Silvestre, Coelho, Póvoa 2010) The lack of association between CRP levels and eventual outcome suggests that it is the degree of systemic response to hepatic injury, rather than the extent of hepatocyte necrosis *per se*, that determines outcome in this condition.

In conclusion, this observational cohort study of acute liver injury patients demonstrates extremely elevated levels of PTX3 in patients with paracetamol-induced acute liver injury. PTX3 correlated with the degree of systemic inflammation and with eventual outcome, including the development of HE and requirement for OLT. Future studies should examine the role of PTX3 in infectious complications of ALF, whilst animal models should explore whether modulation of PTX3 affects important histological and systemic outcomes.

Chapter 7. Conclusions and future directions

ALF is a rare but devastating condition which carries a high mortality rate. Paracetamol-induced hepatotoxicity remains the most common cause of ALF in developed countries including the United Kingdom. Existing prognostic scores for this condition are highly specific, but lack sensitivity, particularly in patients who have taken an unintentional or staggered paracetamol overdose. It is increasingly recognised that the innate immune response to sterile tissue injury, such as paracetamol hepatotoxicity, drives systemic inflammatory responses and results in collateral organ damage. Chapter 4 demonstrates the strong association between the development of the SIRS, extrahepatic organ failure, and eventual outcome following paracetamol overdose. This raises the prospect of using SIRS and SOFA scores as highly sensitive gatekeepers to quantitatively triage paracetamol overdose patients, and deliver tertiary level care to those most likely to require emergency OLT.

A greater understanding of the pathophysiological links between the initial hepatic injury and development of the SIRS could help to identify novel biomarkers for ALF, and help guide future therapeutic avenues. Where possible, biomarkers should be widely available, reproducible between laboratories, and cheap. Serum ferritin fulfils many of these desirable credentials, and chapter 5 demonstrates its potential as an early biomarker of paracetamol overdose. Chapter 6 outlines the strong association between the long pentraxin PTX3, a humoral pattern recognition receptor, with outcome following paracetamol hepatotoxicity, further emphasising the importance of the innate immune response in the pathogenesis of this condition.

Future studies should expand upon the clinical significance of these novel biomarkers by examining their diagnostic and prognostic utility in external cohorts of paracetamol-induced acute liver injury patients, and also in toxicology centres to determine whether these markers remain valid in populations where the majority of patients sustain only minor organ injury. Cellular and animal-based studies should attempt to further unravel the links between the initial hepatotoxic insult and the innate immune response to this injury.

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Appendix

The work described in this thesis has resulted in the following publications:

Craig DG, Lee A, Hayes PC, Simpson KJ: **'Review article: current management of acute liver failure'** *Aliment Pharm & Ther* 2009; 31(3):345-58

Craig DG, Ford AC, Hayes PC, Simpson KJ: **'Systematic review: Prognostic tests of paracetamol-induced acute liver failure'** *Aliment Pharm & Ther* 2010; 31(10):1064-76

Craig DG, Bates CM, Davidson JS, Martin KG, Hayes PC, Simpson KJ:**'Overdose pattern and outcome in paracetamol-induced acute severe hepatotoxicity'** *British Journal of Clinical Pharmacology* 2011; 71(2):273-82

Craig DG, Reid TW, Martin KG, Davidson JS, Hayes PC, Simpson KJ:' **The systemic inflammatory response syndrome and sequential organ failure assessment scores are effective triage markers following paracetamol (acetaminophen) overdose'** *Aliment Pharm & Ther* 2011;34(2):219-28

Craig DG, Lee P, Pryde A, Walker SW, Beckett GJ, Hayes PC, Simpson KJ: **'The long pentraxin PTX3, but not hepatocyte derived CRP, are associated with adverse outcomes following paracetamol overdose'** Oral presentation, British Association for the Study of the Liver Annual Meeting, Edinburgh, 2010

Craig DG, Bates CM, Hayes PC, Simpson KJ: **'Accidental paracetamol overdose and outcome in acute severe hepatotoxicity: a cohort study'** Poster presentation, Scottish Society of Gastroenterology Annual Meeting, Dundee, 2009

Craig DG, KJ Simpson, PC Hayes: **'Predictors of spontaneous survival in fulminant hepatic failure patients meeting King's College Criteria'** Poster presentation, European Association for the Study of the Liver Annual Meeting, Copenhagen, 2008

Alimentary Pharmacology & Therapeutics

Review article: the current management of acute liver failure

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Publication data
Submitted 17 September 2009
First decision 8 October 2009
Resubmitted 15 October 2009
Accepted 15 October 2009
Epub Accepted Article 21 October 2009

SUMMARY

Background
Acute liver failure is a devastating clinical syndrome with a persistently high mortality rate despite critical care advances. Orthotopic liver transplantation (OLT) is a life-saving treatment in selected cases, but effective use of this limited resource requires accurate prognostication because of surgical risks and the requirement for subsequent life-long immunosuppression.

Aim
To review the aetiology of acute liver failure, discuss the evidence behind critical care management strategies and examine potential treatment alternatives to OLT.

Methods
Literature review using Ovid, PubMed and recent conference abstracts.

Results
Paracetamol remains the most common aetiology of acute liver failure in developed countries, whereas acute viral aetiologies predominate elsewhere. Cerebral oedema is a major cause of death, and its prevention and prompt recognition are vital components of critical care support, which strives to provide multiorgan support and 'buy time' to permit either organ regeneration or psychological and physical assessment prior to acquisition of a donor organ. Artificial liver support systems do not improve mortality in acute liver failure, whilst most other interventions have limited evidence bases to support their use.

Conclusion
Acute liver failure remains a truly challenging condition to manage, and requires early recognition and transfer of patients to specialist centres providing intensive, multidisciplinary input and, in some cases, OLT.

Aliment Pharmacol Ther **31**, 345–358

doi:10.1111/j.1365-2036.2009.04175.x

INTRODUCTION

Acute liver failure (ALF) is a rare disorder characterized by catastrophic loss of liver cell function. It remains one of the most challenging medical emergencies, because of the multiorgan nature of the disease, the rapid evolution of the clinical condition, the need for multidisciplinary supportive interventions and the requirement for the clinician to prognosticate accurately to best utilize orthotopic liver transplantation (OLT) as a life-saving treatment. Despite advances in supportive care, spontaneous survival without OLT is as low as 20%; therefore, early recognition and prompt transfer of potential transplant candidates to tertiary centres with intensive care and liver transplantation expertise are vital.

DEFINITION

Acute liver failure refers to the abrupt loss of hepatic cellular function in a patient without pre-existing liver disease, with the subsequent development of coagulopathy, jaundice and encephalopathy. In 1970, Trey and Davidson defined fulminant hepatic failure as a 'potentially reversible condition, the consequence of severe liver injury, with an onset of encephalopathy within 8 weeks of the appearance of the first symptoms and in the absence of pre-existing liver disease'.[1] However, as the initial symptoms of liver failure are frequently nonspecific and open to subjective bias, ALF was redefined based on the time taken to develop hepatic encephalopathy (HE) after the first appearance of jaundice, with the terms 'hyperacute', 'acute' and 'subacute' liver failure referring to a jaundice-to-encephalopathy interval of 0–7, 8–28 and 29–84 days respectively.[2] This distinction is useful in guiding prognosis as, paradoxically, the time to onset of encephalopathy is negatively correlated with outcome despite the increased incidence of cerebral oedema in hyperacute liver failure. Hepatitis A and B, paracetamol and ischaemic insults typically present as hyperacute liver failure, and have a relatively good spontaneous survival rate of 36%, whereas idiosyncratic drug reactions and indeterminate causes present later, with only a 14% survival rate without OLT.[3] Severe acute liver injury, with elevated transaminases and coagulopathy, typically precedes hyperacute liver failure but it is HE, the cardinal feature of the progression to ALF, which defines the condition and is of major prognostic significance.

INCIDENCE AND AETIOLOGY

The accurate reporting of ALF is hampered by the heterogeneous nature of the syndrome and by the lack of an International Classification of Diseases code for ALF. This means that the incidence is probably underreported but has been estimated at 2800 cases per annum in the US or approximately 3.5 deaths per million population.[4, 5] Within Scotland, the incidence of paracetamol-induced ALF has been estimated at 8.4 cases per annum per million population.[6] The syndrome of ALF is not a single clinical entity, and may be precipitated by a wide variety of hepatic insults (Table 1).[7] These insults have a marked geographical and socioeconomic variation, with the most common aetiologies in Europe and North America being paracetamol and idiosyncratic drug reactions, whereas developing countries have a higher preponderance of acute viral aetiologies (Table 2). Specific insults also demonstrate geographical variation, with staggered accidental paracetamol overdoses predominating in the US, whereas single, intentional, overdoses are more common in the UK.[8, 9] The early identification, where possible, of the underlying aetiology of ALF is crucial as several causes of ALF, such as paracetamol (*N*-acetyl cysteine, NAC), *Amanita phalloides* poisoning (penicillin and silibinin), fulminant hepatitis B (lamivudine), herpes simplex virus (HSV) (acyclovir) and pregnancy (delivery), have specific treatments and the prognosis between different aetiologies varies considerably.[10–14]

CLINICAL FEATURES

The initial clinical features of ALF may be nonspecific and may include anorexia, fatigue, abdominal pain, jaundice and fever before progressing to HE.[15] The 'type A' HE associated with ALF (types B and C HE are associated with portosystemic bypass and cirrhosis respectively) is graded from 1 to 4 on clinical features and neurological signs (Table 3).[16] This grading is clinically robust, and increasing grades of HE have a strong negative correlation with outcome. The pathogenesis of HE (and subsequent cerebral oedema) in ALF is incompletely understood, and differs from the encephalopathy observed in chronic liver disease, but hyperammonaemia, systemic inflammation and loss of cerebral blood flow (CBF) autoregulation all appear to accelerate progression.[17–19] Ammonia, produced from glutamine by enterocytes, enters the systemic

Table 1. Selected aetiologies of acute liver failure

Aetiological category	Specific causes
Viral	HAV, HBV +/− HDV, HEV, HSV, human herpes virus 6, CMV, EBV, VZV, parvovirus B19, yellow fever
Drug/toxin induced (dose-dependent)	Paracetamol, *Amantina phalloides*, tetracyclines, *Bacillus cereus*, CCl_4
Drug/toxin induced (idiosyncratic)	Halothane, anti-tuberculous therapy, sulphonamides, coamoxiclav, macrolides, valproate, NSAIDs, disulfiram, thalidomide, β-interferon, HAART, Ecstasy, cocaine, herbal remedies, etc.
Vascular	Ischaemic hepatitis, Budd–Chiari, right heart failure, veno-occlusive disease
Metabolic	Wilson's disease, acute fatty liver of pregnancy, HELLP
Miscellaneous	Autoimmune hepatitis, malignant infiltration, sepsis, heat stroke
Others	Cryptogenic

HAV, hepatitis A virus; HBV, hepatitis B virus; HDV, hepatitis delta virus; HEV, hepatitis E virus; HSV, herpes simplex virus; CMV, cytomegalovirus; EBV, Epstein–Barr virus; VZV, Varicella zoster virus; HLLP, haemolysis, elevated liver enzymes and low platelets; HAART, Highly Active Anti-Retroviral Treatment.

circulation via the portal vein, but is poorly cleared by the failing liver, and this is exacerbated by the coexistent renal failure, reduced hepatic urea synthesis and impaired skeletal muscle function observed in ALF.[20] Astrocytes detoxify ammonia by converting it to glutamine which leads to osmotic swelling; this cytotoxic process appears to be the main pathophysiological driver of cerebral oedema in ALF.[3, 21, 22] Hyponatraemia, frequently seen in patients with ALF, may potentiate this process, possibly via aquaporin-4.[23, 24] In additio, cerebral hyperaemia, potentially mediated by pro-inflammatory cytokines, increases intracranial blood volume, further compromising cerebral perfusion.[25, 26] Interestingly, case reports have directly implicated liver-derived toxins in this process.[27]

The evolution to grade III/IV HE is a grave prognostic sign as this group is at risk of intracranial hypertension and subsequent brain herniation.[28] In addition, intracranial hypertension compromises cerebral perfusion pressure (CPP), defined as the difference between mean arterial pressure and intracranial pressure (ICP). A CPP >60 mmHg is considered crucial to maintain normal neurological functioning and periods >2 h with a CPP <40 mmHg is considered by some centres to preclude liver transplantation.[5] Clinical signs suggestive of increasing ICP include worsening of HE, systemic hypertension and bradycardia (Cushing reflex), altered pupillary reflexes and decerebrate rigidity. All of these clinical signs occur late in the clinical course, when therapeutic interventions may be ineffective, and this has led to the direct monitoring of ICP in patients with ALF.

Intracranial pressure monitoring

The use of ICP monitoring in ALF remains controversial, because of the lack of consensus over treatment goals, the associated risks of bleeding and infection and the lack of randomized trial data supporting improved survival. However, continuous monitoring permits rapid, targeted treatment to be initiated and several groups now include ICP monitoring as part of their standard ALF protocol, particularly in potential OLT candidates.[5, 29] Intracerebral bleeding occurs in 8–10% of cases, although fatal bleeds occur considerably less frequently.[30, 31] Calculation of cerebral oxygen consumption using a jugular bulb catheter may provide additional information through continuous, indirect assessment of CBF.[32] This involves the retrograde passage of a fine-bore catheter into the jugular vein until the tip reaches the jugular bulb; venous saturations >85% (normal range 55–70%) represent a hyperaemic cerebral circulation.

Treatment for raised ICP

The goal of medical management of cerebral oedema should be to maintain the ICP <20 mmHg and the CPP >70 mmHg, by reducing brain volume or CBF. Basic manoeuvres include elevation of the head of the bed to no more than 30° and minimizing painful stimuli including suctioning.[33] Hyperventilation produces, at best, a transient restoration of CBF autoregulation by lowering $PaCO_2$; however, its prolonged use in ALF patients has been questioned.[34] Established therapies

Table 2. Aetiologies of acute liver failure by geographical location

Country	UK*	US	Canada	Scandinavia	France	Spain	Chile†	Australasia	Sudan	India	
Reference	138	139	140	141	142	143	144	145	146	147	
No. cases	310	308	81	315	363	267	27	80	37		180
Years	1994–2004	1998–2001	1991–1999	1990–2001	1986–2006	1992–2000	1995–2003	1988–2001	2003–2004	1989–1996	
Paracetamol (%)	43	39	15	17	7	2	0	36	0		0
Nonparacetamol drug reactions (%)	8	13	12	10	21	14	7	6	8		0.6
Hepatotropic viruses (%)	7	12	30	12	33	37	37	14	27	68 (44 Hep E)	
Indeterminate (%)	30	17	27	43	18	32	44	34	38		31
Other causes (%)	12	19	16	17	21	15	11	10	27		0

* Patients listed for orthotopic liver transplantation only.
† Paediatric patients only.

for raised ICP include the use of mannitol and barbituates such as thiopentone.[35, 36] More limited evidence supports the use of hypertonic saline, propofol sedation and indomethacin.[37, 38] Unrandomized studies from Edinburgh have advocated the use of moderate hypothermia (32–33 °C) in advanced ALF as a means of reducing ICP prior to, and during, transplantation, and this appears to offer a therapeutic option which targets many of the proposed triggers for elevated ICP in ALF.[20, 39–41] Further studies are required to clarify the optimal extent and duration of hypothermia and to exclude a negative impact from hypothermia upon sepsis, coagulopathy and cardiac stability.

HAEMODYNAMIC, RESPIRATORY AND RENAL INSTABILITY

Acute liver failure is characterized by a hyperdynamic circulation, with markedly reduced systemic vascular resistance, increased cardiac output and hypotension, which frequently necessitates vasopressor support in addition to fluid repletion.[42] As yet, the optimal fluid and vasopressor strategy remains uncertain, but most centres use crystalloid resuscitation followed by norepinephrine infusions to maintain adequate perfusion pressures and cerebral oxygenation, although this strategy may not significantly improve oxygen delivery.[43] Overzealous norepinephrine use should be avoided as this may exacerbate cerebral hyperaemia because of the loss of CBF autoregulation in ALF.[44] Terlipressin, a vasopressin synthetic analogue, has been evaluated in two small studies which produced conflicting results regarding its effects on CBF, ICP and systemic haemodynamic parameters.[45, 46]

Critical illness-related corticosteroid insufficiency (CIRCI) may exacerbate the haemodynamic instability seen in ALF, with a negative correlation between illness severity and the response to short synacthen testing in a cohort of 45 patients with acute liver injury.[47] Corticosteroid treatment of patients with low baseline cortisol levels ('hepatoadrenal syndrome') in a liver intensive care unit resulted in reduced vasopressor requirements and mortality, but it is worth noting that adrenal insufficiency was commoner in patients with chronic liver disease or post-OLT in this study than in the ALF patients.[48] There is insufficient evidence at this stage to recommend routine treatment of CIRCI in liver patients,

Table 3. Classification of hepatic encephalopathy[148]

HE Grade	Mental status	Neurological signs	EEG	GCS
I	Trivial lack of awareness; euphoria or anxiety; shortened attention span; impairment of addition/subtraction	Slight tremor; apraxia; incoordination	Usually normal	15
II	Lethargy or apathy; disorientation for time; obvious personality change; inappropriate behaviour	Asterixis; ataxia; dysarthria	Generalized slowing	11–15
III	Somnolence to semi-stupor; responsive to stimuli; confused; gross disorientation; bizarre behaviour	Asterixis; ataxia	Abnormal	8–11
IV	Coma; unable to test mental state	Decerebration	Abnormal	<8

EEG, electroencephalogram; GCS, glasgow coma scale.

and the use of synacthen tests to diagnose CIRCI is not recommended.[49]

Radiographic changes suggestive of pulmonary oedema occur in a high proportion of ALF cases, and the development of severe acute lung injury is associated with a poor prognosis.[50, 51] Treatment of ARDS using a protective ventilatory strategy is more problematic in ALF because increases in positive end expiratory pressure may exacerbate cerebral oedema and hepatic congestion.[7]

Renal failure in ALF is multifactorial, and is related to acute tubular necrosis, hypoperfusion, use of contrast agents and, in paracetamol-induced ALF, direct nephrotoxicity.[52] The systemic inflammatory response syndrome (SIRS) has recently been shown to predict renal dysfunction in nonparacetamol-induced ALF.[53] Management should focus on prevention of renal failure by maintaining adequate systemic blood pressure, prompt identification and treatment of infections and judicious use of contrast agents, because once established, the prognosis is considerably poorer. Continuous, rather than intermittent, methods of extracorporeal support are preferred to minimize circulatory and cerebral fluctuations.[54] Hypophosphataemia is associated with renal failure in ALF and may also be linked to hepatic regeneration; persistently elevated phosphate levels may reflect failure of this regenerative process and have been associated with a poorer prognosis in paracetamol-induced ALF.[55, 56] Reduced hepatic glycogen stores and hyperinsulinaemia contribute to hypoglycaemia, which complicates up to 40% of ALF cases, and continuous glucose administration is frequently required. Oral or enteral feeding is vital, but the markedly increased energy expenditure observed in ALF makes adequate nutritional support difficult to institute effectively in established liver failure.[57]

COAGULOPATHY

The coagulopathy of ALF is complex and remains poorly characterized, but is undoubtedly of prognostic significance.[58–60] ALF is characterized by prolongation of prothrombin time and quantitative and qualitative platelet dysfunction and, in paracetamol-induced ALF, hypofibrinogenaemia and reductions in coagulation factors II, V, VII and X.[61] However, despite the severity of the coagulopathy, clinically significant spontaneous bleeding is relatively unusual in ALF, even during liver transplantation.[62, 63] The defective production of procoagulant factors is compensated for in part by underproduction of anticoagulant proteins protein C, protein S and antithrombin III, whilst factor VIII production is upregulated, possibly in extrahepatic sites.[64, 65] Plasminogen activator inhibitor-1 levels are grossly elevated in ALF, suggesting hypofibrinolysis; this was supported by a recent murine study where heparin pre-treatment significantly reduced hepatic fibrin deposition following paracetamol poisoning and significantly attenuated paracetamol-induced hepatotoxicity.[66, 67] The bleeding risk in ALF has been overstated, and the prophylactic administration of large volumes of fresh frozen plasma (FFP) in ALF is unnecessary, interferes with prognostic scoring systems and may worsen cerebral oedema or volume overload.[68, 69] Several pilot studies have suggested that recombinant

factor VII may be superior to FFP for clinically significant bleeding in ALF, but further evaluation is required before this expensive treatment can be universally recommended.[70, 71] At present, FFP, platelet and cryoprecipitate infusions should be reserved for use in actively bleeding patients or prior to planned invasive procedures.[72]

INFECTION

The SIRS and, when precipitated by infection, sepsis have been shown to have a strong negative impact on HE progression, renal function and mortality in ALF.[19, 53, 73] Bacteraemia is present in up to 80% of ALF patients who have enhanced susceptibility to infection because of the presence of indwelling lines and catheters, impaired complement and opsonization function and impaired innate immunity.[74] The majority of infections are caused by Gram-negative enteric organisms, staphylococci and fungal infections.[75, 76] The role of bacterial gut translocation, important in cirrhosis, is unclear at present, as prospective randomized trials of oral and enteral gut decontamination in ALF have failed to add additional benefit over parenteral antibiotic regimens alone.[77–79] Fungal infections are commonly underrecognized and are particularly important in ALF patients who have received prolonged courses of antibiotics or have renal dysfunction.[76] Close surveillance for infection should be maintained in all ALF patients with frequent chest radiographs and cultures of blood, urine and sputum, but prophylactic antibiotics should probably be reserved for patients with high-grade encephalopathy and renal dysfunction or for those awaiting OLT.[69, 78]

SPECIFIC THERAPIES IN ALF

N-acetyl cysteine

When used early as an antidote after a single, intentional paracetamol overdose, NAC is extremely effective at replenishing hepatic glutathione stores and preventing severe *N*-acetyl-p-benzoquinone imine-induced hepatotoxicity and liver failure.[10] The evidence for NAC in patients with established hepatotoxicity or ALF is less robust, and is based on a retrospective study from King's College Hospital and a small randomized controlled trial from the same centre.[80, 81] Initial studies suggested that NAC improved oxygen delivery and consumption in ALF, but this assertion has subsequently been challenged.[82, 83] Furthermore, the optimal duration of NAC therapy in these patients is unclear, as prolonged NAC therapy was recently shown to impair murine liver regeneration and worsen outcome following paracetamol poisoning.[84] The benefit of NAC in nonparacetamol-induced ALF is also unclear, and although it appears to confer benefit in children, a recent multicentre randomized-controlled trial (RCT) in adults only demonstrated improvement in spontaneous survival in a subgroup of patients with Grade I–II HE.[85, 86]

Penicillin and silibinin

Mycetismus (mushroom poisoning), most commonly from *Amanita* genus mushrooms, is a medical emergency. Amantinin toxin, recycled via the enterohepatic circulation, interrupts hepatocyte messenger RNA synthesis and causes dose-dependent hepatotoxicity. Initial promising reports describing charcoal haemoperfusion in the treatment of *Amanita* poisoning have not been replicated.[87] Penicillin G (250 mg/kg/day) and silibinin (20–50 mg/kg/day), although never subjected to an RCT, appear to be effective when administered early in the course of the disease, but severe cases may require emergency OLT.[11, 88]

Lamivudine

A small proportion of patients with acute hepatitis B proceed to develop ALF, which occasionally necessitates OLT. Previous case reports utilized foscarnet in the treatment of fulminant hepatitis B, but increasing attention is being given to nucleoside analogues in this condition.[13, 89–91] However, an RCT of lamivudine involving 71 patients with acute hepatitis B (three of whom had HE) failed to demonstrate any significant clinical benefit in the lamivudine arm.[12]

Delivery of pregnant ALF patients

The syndrome of haemolysis, elevated liver enzymes and low platelets (HELLP) and acute fatty liver of pregnancy (AFLP) are the pregnancy-related liver disorders most commonly associated with ALF, although pre-eclampsia can occasionally result in hepatic rupture and infarction. There is increasing evidence that pre-eclampsia, HELLP and AFLP represent a spectrum of the same disease, with similar clinical and pathophysiological correlations including increased vascular

tone and platelet aggregation.[92] Foetal deficiency of the mitochondrial enzyme long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) results in maternal accumulation of medium- and long-chain fatty acids, and similar mutations of the mitochondrial trifunction protein have been linked to both HELLP and AFLP.[93–95] Pre-eclampsia, HELLP and AFLP all carry significant risk of both maternal and foetal mortality, and severe cases should be managed in tertiary centres capable of dealing with the potential obstetric and hepatic complications. The mainstay of treatment of all the three conditions is delivery, but close postpartum observations of both mother and infant are important to detect any haemorrhagic complications or continued clinical or biochemical deterioration, which can occasionally necessitate postpartum emergency OLT.[96–99]

Acyclovir

Whilst HSV has high seroprevalence amongst the general population, HSV-related hepatitis is a rare cause of ALF (1.4% in one 13-year cohort study) and is most commonly, but not exclusively, seen amongst immunosuppressed and pregnant patients.[100] The diagnosis of HSV-ALF is frequently made late, with a resultant high mortality rate. As the characteristic vesicles are often absent, a high index of suspicion must be maintained to screen for potential cases amongst young, immunosuppressed, or pregnant individuals presenting with fever and transaminitis, as prompt treatment with intravenous acyclovir is safe and has shown benefit in several cases.[14, 101]

Plasmapheresis and D-penicillamine

Fulminant Wilson's disease represents a rare, but important cause of ALF, and the diagnosis can be intimated by the presence of a low alkaline phosphatase to bilirubin ratio, AST:ALT ratio >2.2 and haemolytic anaemia.[102, 103] Isolated cases report the successful use of plasmapheresis and chelation therapy in the treatment of fulminant Wilson's disease, although these remain a bridge to transplantation rather than definitive therapies.[104, 105] Plasmapheresis appears to reduce arterial ammonia levels, and has occasionally been utilized in the treatment of aetiologies of ALF other than Wilson's disease, but the side effects of treatment can be significant and no mortality benefit has been demonstrated to date.[106–108]

Other treatments

Other treatments such as insulin/glucagon, prostaglandin E2 and corticosteroids (even in fulminant autoimmune hepatitis) have failed to show significant benefit in ALF and are not recommended.[82, 109–112] A recent RCT of L-ornithine–L-aspartate (LOLA) in ALF patients, predominantly with acute viral hepatitis, found LOLA to be ineffective in reducing either ammonia levels or mortality rates, with a trend towards increased seizure rates in the LOLA arm.[113]

Artificial and bioartificial liver devices

Liver assist devices have received much attention over recent years in the hope that they can provide an effective 'bridge' to transplantation or recovery of liver function. The initial artificial liver support devices were essentially filters designed to remove toxins through haemodialysis or adsorption using charcoal, and failed to show a survival benefit in ALF.[114] The MARS (Molecular Absorbent and Recirculating System) utilizes a hollow fibre, double-sided, albumin-impregnated dialysis membrane to extract protein-bound toxins into the albumin dialysate.[108] Initial reports suggested improvements in both systemic and cerebral haemodynamic parameters and improvements in HE in patients with ALF and acute-on-chronic liver failure.[115–118] More advanced systems, such as Prometheus, involve fractionated plasma separation and adsorption, whilst bioartificial liver systems include living human or porcine hepatocytes incorporated in an extracorporeal circuit to add synthetic function to the process of detoxification. At least 12 RCTs of these devices have been performed and they have been systematically reviewed twice; overall, these devices improve HE but have no mortality benefit in ALF, but may improve outcome in acute-on-chronic liver failure.[119, 120]

Liver transplantation

Although never subjected to an RCT, OLT has been recommended for the treatment of ALF since 1983.[121] Emergency OLT for ALF now accounts for 5–12% of all liver transplantation activity and is the only treatment to date to alter substantially the mortality resulting from the condition.[122] Patient survival following OLT for ALF is generally poorer than that in those transplanted for chronic liver failure, particularly in

the setting of pre-transplant renal failure, but is of the order of 65–80% 1 year survival.[123–125] Alternatives to standard OLT are being refined, including living donor grafts and auxiliary liver transplantation (where part of the native liver is left *in situ* after partial liver transplantation in the hope that native liver regeneration can permit later cessation of immunosuppression).[126, 127] Whilst a promising therapy for paracetamol-induced ALF, auxiliary transplantation fails to remove the diseased native liver, leading to concerns over continuing haemodynamic and neurological instability. The limited timescales and the dramatic nature of the disease progression involved mean that the possibility of coercion of potential living donors in the setting of ALF is of significant ethical concern. In addition, ALF patients are at particular risk of small-for-size syndrome and therefore the larger right lobe from the donor is usually transplanted, increasing the risk of donor morbidity or mortality.[128]

PROGNOSTICATION TO GUIDE OLT IN ALF

The decision to list emergently an ALF patient for OLT is rarely easy – the inherent risks associated with delaying listing for OLT must be balanced against the potential for spontaneous recovery with medical therapy alone, the risks of surgery in the context of an acute critical illness, the scarcity of donor grafts and the requirement (except after auxiliary liver transplantation) for life-long immunosuppression.[127] Furthermore, up to 60% of paracetamol-induced ALF patients meeting poor prognostic criteria are deemed unsuitable to undergo OLT because of coexistent psychiatric or medical conditions that are likely to preclude long-term graft and/or patient survival, such as resistant alcohol or drug dependence, or previous persistent treatment noncompliance.[129] Accurate prognostication in ALF is therefore vital, and many proposed mathematical, serological, radiological and histological variables have been proposed, including the MELD (Model for End-stage Liver Disease) score, which has improved organ allocation in chronic liver disease (Table 4).[130, 131] Major methodological flaws exist with many of these studies, which are often unblinded, retrospective and prone to spectrum bias.[132] Furthermore, many authors equate liver transplantation with death, falsely elevating the positive predictive value of the test in question.[133] In 1989, O'Grady *et al.* developed the 'King's College criteria' from a retrospective cohort of 588 patients with grade II–IV HE admitted to that

Table 4. Alternative prognostic variables suggested for use in acute liver failure

Prognostic variable	Aetiology	Predictor of poor prognostic outcome	Sensitivity	Specificity	Refs
KCC	All	See Table 5	69	92	58
Clichy criteria	All	HE + Factor V < 20% (age <30 year)	–	–	149
		or <30% (age >30 year)	86	76	60
		Grade III–IV HE + Factor V < 20%			
Factor V; factor VIII/V ratio	Paracetamol	Factor VIII/V ratio >30	91	91	150
		Factor V < 10%	91	100	
Phosphate	Paracetamol	PO_4^{3-} > 1.2 mmol/L on day 2 or 3 postoverdose	89	100	56, 151
APACHE II	All	APACHE II >19	68	87	8
Gc-globulin	All	Gc-globulin <100 mg/L	73	68	152
		Paracetamol	30	100	
		Nonparacetamol			
Lactate	Paracetamol	Admission arterial lactate >3.5 or >3.0 mmol/L after fluid resuscitation	81	95	134
α-Fetoprotein	Paracetamol	AFP <3.9 μg/L 24 h postpeak ALT	100	74	153
MELD	Paracetamol	MELD >33 at onset of HE	60	69	154
	Nonparacetamol	MELD >32	76	67	155

KCC, King's College Hospital poor prognostic criteria; MELD, Model for End-stage Liver Disease; HE, hepatic encephalopathy; APACHE II, Acute Physiology and Chronic Health Evaluation II; AFP, alpha fetoprotein.

Table 5. The KCH poor prognostic criteria for paracetamol and nonparacetamol acute liver failure aetiologies as applied in the UK as transplant criteria[58, 134]

Paracetamol	Modified criteria
List for transplantation if: Arterial pH <7.3	Strongly consider listing for OLT if: Arterial lactate >3.5 mmol/L after early fluid resuscitation
Or all 3 of the following occur within a 24-h period: Grade III–IV HE PT >100 s (INR >6.5) Serum creatinine >300 μmol/L	List for transplantation if: Arterial pH <7.3, or arterial lactate >3.0 mmol/L after adequate fluid resuscitation List for transplantation if all 3 of the following occur within a 24-h period: Grade III–IV HE PT >100 s (INR >6.5) Serum creatinine >300 μmol/L
Nonparacetamol	
List for transplantation if: PT >100 s (INR >6.5) irrespective of HE grade Or any 3 of 5 of the following: Unfavourable aetiology: (seronegative hepatitis, Wilson's disease, idiosyncratic drug reaction, halothane) Age <10 or >40 years Jaundice to encephalopathy interval >7 days PT >50 s (INR >3.5) Bilirubin >300 μmol/L	

HE, hepatic encephalopathy; OLT, orthotopic liver transplantation; PT, prothrombin time.

institution.[58] The criteria recognize the prognostic importance of the aetiology of ALF, with separate criteria for paracetamol-induced ALF and other aetiologies (Table 5). These criteria are remarkable for their robustness and accuracy despite numerous attempts to improve their diagnostic accuracy (Table 4). The paracetamol criteria have recently been modified by the addition of lactate, although several authors have questioned the overall benefit of this modification.[134–136] The King's College criteria have also been criticized for its low sensitivity and negative predictive value, particularly for nonparacetamol aetiologies.[137]

CONCLUSIONS

Acute liver failure remains a truly challenging condition to manage, and requires close surveillance for incipient organ failure and early transfer of patients to specialist centres offering intensive multidisciplinary input to manage effectively the myriad complications of the syndrome, and, in some cases, to offer liver transplantation. The rarity of the syndrome presents difficulties in performing RCTs and developing a true evidence base for many of the interventions outlined in this review. The future challenges are to further elucidate the pathophysiology behind liver cell death and the ensuing multiorgan failure of ALF, which in turn may help improve prognostic models. Further multicentre controlled trials of artificial and bioartificial liver support systems are needed before recommendations can be made regarding their clinical utility in ALF. Effective reduction of ammonia levels remains an attractive, but currently elusive, therapeutic goal in ALF.

ACKNOWLEDGEMENT

Declaration of personal and funding interests: None.

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Alimentary Pharmacology & Therapeutics

Systematic review: prognostic tests of paracetamol-induced acute liver failure

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Publication data
Submitted 15 January 2010
First decision 14 February 2010
Resubmitted 20 February 2010
Accepted 21 February 2010
Epub Accepted Article 23 February 2010

This uncommissioned systematic review was subject to full peer-review.

SUMMARY

Background
Paracetamol (acetaminophen) toxicity remains the leading cause of acute liver failure (ALF) in the developed world. In the UK, the recently modified King's College Criteria are used to list patients for emergency liver transplantation, but these criteria have been criticized for their low sensitivity and for spectrum bias in their application.

Aim
To evaluate existing prognostic criteria critically for predicting death without transplantation in paracetamol-induced ALF.

Methods
MEDLINE, EMBASE and CINAHL were searched to identify studies containing adult patients with paracetamol-induced ALF. Selected studies were evaluated and data were pooled if appropriate, to calculate sensitivity, specificity and diagnostic odds ratios (DORs) of applied prognostic tests.

Results
Of 6507 studies identified, 14 were eligible for inclusion, evaluating 1960 patients. The original King's College Criteria had a pooled sensitivity of 58.2% and specificity of 94.6%, with a DOR of 27.7. Addition of arterial lactate to the King's College Criteria reduced the DOR to 26.1. Several other clinical and laboratory variables had higher DORs than the King's College Criteria, but were only evaluated in single studies of limited quality.

Conclusions
The original King's College Criteria remain well-validated criteria with high prognostic accuracy. Other potential prognostic variables should be prospectively assessed in multicentre studies to refine the criteria further.

Aliment Pharmacol Ther **31**, 1064–1076

doi:10.1111/j.1365-2036.2010.04279.x

INTRODUCTION

Paracetamol (acetaminophen) toxicity remains the leading cause of acute liver failure (ALF) in the developed world, accounting for over 40% of cases in selected case series.[1, 2] Whilst a vast majority of patients recover spontaneously following paracetamol overdose, a small number develop severe acute liver injury, hepatic encephalopathy (HE) and consequently, ALF. Despite significant advances in supportive care, the only effective treatment for the condition remains emergency orthotopic liver transplantation (OLT).[3]

The decision to transplant a patient with paracetamol-induced ALF involves balancing the inherent risks associated with delaying listing for OLT against the potential for spontaneous recovery with medical therapy alone, the risks of surgery in the context of a rapidly evolving critical illness, the scarcity of donor grafts and the requirement for lifelong immunosuppression. Furthermore, the psychosocial implications of paracetamol overdose cases are considerable, with over 30% of patients who fulfil transplant criteria unsuitable for OLT because of severe psychological illness or coexistent chronic alcohol or drug dependency.[4] Accurate prognostication in ALF is therefore vital to utilize OLT effectively and prevent unnecessary transplantation, whilst procuring donor organs in a timely fashion for those most likely to benefit.

In 1989, O'Grady *et al.*[5] developed the 'King's College Criteria' (KCC) in an attempt to determine which patients with paracetamol and nonparacetamol-induced ALF have a poor prognosis with medical therapy alone and will therefore benefit most from OLT. The original KCC for paracetamol-induced ALF were highly specific, but have been criticized for their relatively low negative predictive value[6] and, as up to 26% of patients with paracetamol toxicity are medically unfit to undergo surgery at the point they fulfil the KCC,[4] modifications to these criteria now utilize arterial lactate (>3.5 mmol/L after early fluid resuscitation or >3.0 mmol/L after adequate fluid resuscitation) in an attempt to extend the time-window for acquisition of a suitable graft.[7]

Over recent years, a plethora of alternative prognostic variables have been proposed in an attempt to improve or replace the KCC. These criteria variously involve radiological,[8] histological,[9] serological,[10, 11] or mathematical[12–14] indices, but their assessment is complicated by the use of OLT; for reasons of the inevitably imperfect nature of current listing criteria, a small proportion of ALF patients are transplanted who would have spontaneously survived, invalidating further assessment of that particular patient. Studies evaluating the prognostic accuracy of a particular variable should therefore exclude transplanted patients from subsequent analysis. The purpose of this systematic review was to evaluate critically existing prognostic criteria for predicting death without transplantation in paracetamol-induced ALF, to update previous studies in this area[15, 16] and to examine the accuracy of recently described prognostic variables.

METHODS

Search strategy and study selection

We conducted a systematic review of the medical literature using MEDLINE (1950 to June 2009), EMBASE (1980 to June 2009) and CINAHL (1982 to June 2009) to identify studies recruiting adult (>age 16 years) patients that evaluated prognostic markers of paracetamol-induced ALF. Potential studies were identified by combining the search terms 'acetaminophen' and 'paracetamol' (as both Medical Subject Headings (MeSH) and free text terms) using the set operator OR. Prognostic studies of ALF were identified and combined using the set operator OR, by using the terms 'Kings adj3 College adj3 Criteria', 'Clichy$', 'APACHE$', 'lactate', 'Gc-globulin', 'alpha-fetoprotein', 'phosphate' and 'MELD' (all free text terms) and the McMaster expert search strategy for prognostic studies. These studies were combined using the set operator AND with papers evaluating studies on 'liver failure', 'hepatic encephalopathy' or 'liver failure, acute' (all MeSH). The search was limited to human studies without language restrictions. Abstracts of the studies identified by the initial search were evaluated for appropriateness to the study question by two independent reviewers (DC, KS) and all potentially relevant papers were obtained and evaluated in detail. The bibliographies of these studies and relevant review articles were screened to perform a recursive search of the literature, and abstract books of international liver conferences from the preceding 4 years were hand-searched for potentially relevant studies.

Selected studies were required to report mortality data on cohorts of patients admitted to hospital with acute severe liver injury or ALF secondary to paracetamol overdose (see Table 1 for study eligibility

Table 1. Eligibility criteria for included studies

Adult patients (aged >16 years) presenting with acute severe liver injury or ALF attributed to paracetamol hepatotoxicity
Cohort studies
Included >25 patients with paracetamol hepatotoxicity
Mortality data recorded
Data for death and liver transplantation considered separately

criteria). Acute severe liver injury was defined as severe hepatotoxicity [serum alanine aminotransferase (ALT) levels >1000 U/mL and co-existent coagulopathy], whilst ALF required the additional presence of HE, in patients without pre-existing liver disease. Unselected cohorts of patients with mixed aetiologies of acute liver injury were eligible for inclusion if separate data for paracetamol overdose patients were available. Case control studies and studies comparing ALF patients with chronic liver disease patients were excluded because of potential bias in favour of the prognostic variable in question,[17] as were studies where transplantation and death were combined as a single end-point. Studies were required to include more than 25 patients with paracetamol-induced acute liver injury, as a majority of studies in this area include the KCC as a prognostic variable in addition to the main variable studied, and smaller studies may invalidate analysis.[18] All potential articles were assessed independently by two researchers according to eligibility criteria, which were defined prospectively and disagreements were resolved by consensus.

Data extraction and quality assessment

Data were extracted using predesigned forms by two separate reviewers (DC and KS) on to a Microsoft Excel spreadsheet (Microsoft Corp, Redmond, WA, USA), and any discrepancies were resolved by consensus. The following data were extracted for each study: setting, country and geographical region, year(s) conducted, retrospective or prospective design, inclusion criteria, definitions of paracetamol-induced liver injury, definitions of ALF, total number of subjects included, total number of subjects with paracetamol-induced ALF or acute liver injury, total number of subjects with paracetamol-induced ALF transplanted, duration of follow-up, prognostic score used, timing of application of the prognostic score and outcome definitions. Study quality was assessed semi-quantitatively according to six potential sources of bias that can be encountered in studies of prognostic variables[19] and a grade (poor, moderate, good or excellent) allocated (Table 2).

Data synthesis and analysis

Prognostic tests evaluating the outcomes of survival without transplantation or death without transplantation following paracetamol-induced liver injury were assessed individually. From each study cohort, we extracted total number of subjects dying without OLT, total number of subjects surviving without OLT and total number of subjects fulfilling or not fulfilling the prognostic variable in question, and constructed 2×2 contingency Tables. The sensitivity, specificity and diagnostic odds ratio (DOR) with their 95% confidence

Table 2. Quality assessment of included studies (adapted from Hayden *et al.*[19])

Study population	Study population adequately represents the population of interest; source population adequately described; sampling timeframe, place and period of recruitment described; inclusion and exclusion criteria described
Follow-up	Adequate study completion rate; loss to follow-up not associated with key characteristics; reasons for loss to follow-up described; no important differences between participants completing/not completing study
Prognostic factor	Prognostic measure clearly defined; continuous data reported and any cut-points described and appropriate; method and setting of measurement of prognostic factor identical for all patients; adequate proportion of study population has prognostic factor measured
Outcome measurement	Clear definition of outcome measure of interest described and recorded, including duration of follow-up; outcome measure valid; outcome measure identical
Confounding	All important confounders measured and accounted for at all stages of study design, performance and analysis
Analysis	Sufficient presentation of data to permit assessment of analytical analysis; appropriate model-building, study design etc.; no selective reporting of results

intervals (CIs) were calculated for each test using Microsoft Excel (Microsoft Corp) and checked using Meta-DiSc version 1.4 (Universidad Complutense, Madrid, Spain). The value of a DOR ranges from zero to infinity, with higher values indicating better discriminatory test performance and is calculated from the following formula: sensitivity/(1 − sensitivity)/(1 − specificity)/specificity.[20] Where required, a zero cell correction of 0.5 was added to all cells to prevent computational problems arising where proportions were equal to zero.[21] Where two or more studies analysed a particular prognostic marker, data were pooled using a random effects model and the pooled DOR calculated. Heterogeneity between studies was assessed using the I^2 statistic with a cut off of 50%, and the χ^2 test with a P-value <0.10, used to define a statistically significant degree of heterogeneity. We explored study setting and study design as potential reasons for heterogeneity.[22] These are exploratory analyses only and the results should therefore be interpreted with caution.

RESULTS

The search strategy identified 6507 studies of which 105 were potentially eligible for inclusion and were retrieved for further analysis (Figure 1). Of these, 14 were considered eligible for inclusion[5, 7, 10, 23–33] including two studies published in abstract form only.[32, 33] Characteristics of included studies are provided in Table 3. Cohen's kappa test for inter-observer agreement was excellent at 0.86 (95% CI 0.75–0.98).

The eligible studies evaluated a total of 1960 patients with paracetamol-induced acute liver injury or ALF. Three[10, 24, 27] studies had complete temporal overlap with other studies from the same unit; however, these studies were included for their evaluation of unique prognostic markers separate to the KCC. There was only one multicentre study.[29] Five[25, 26, 28, 31, 32] studies evaluated patient cohorts retrospectively, whereas five[10, 26, 27, 30, 33] studies developed prognostic test thresholds retrospectively [usually from receiver operator characteristics (ROC) curve analysis]. No study blinded observers to patient outcome or other potentially confounding prognostic data and only two[5, 7] validated their prognostic marker in a separate cohort. Consequently, six[24–26, 31–33] of the 14 studies were graded as poor and seven[5, 7, 10, 23, 28–30] as moderate quality (Table 3). The 14 eligible studies analysed a total of 22 different prognostic markers or variations thereof. The sensitivity, specificity and DORs of these are reported in Table 4.

King's college criteria

A total of 13 studies evaluated the original KCC for paracetamol-induced ALF, either as separate elements [arterial pH <7.3 or concurrent grade III/IV HE, serum creatinine >300 μmol/L and prothrombin time (PT) >100 s][5, 25] or as a whole,[7, 10, 23, 24, 26–32] including a

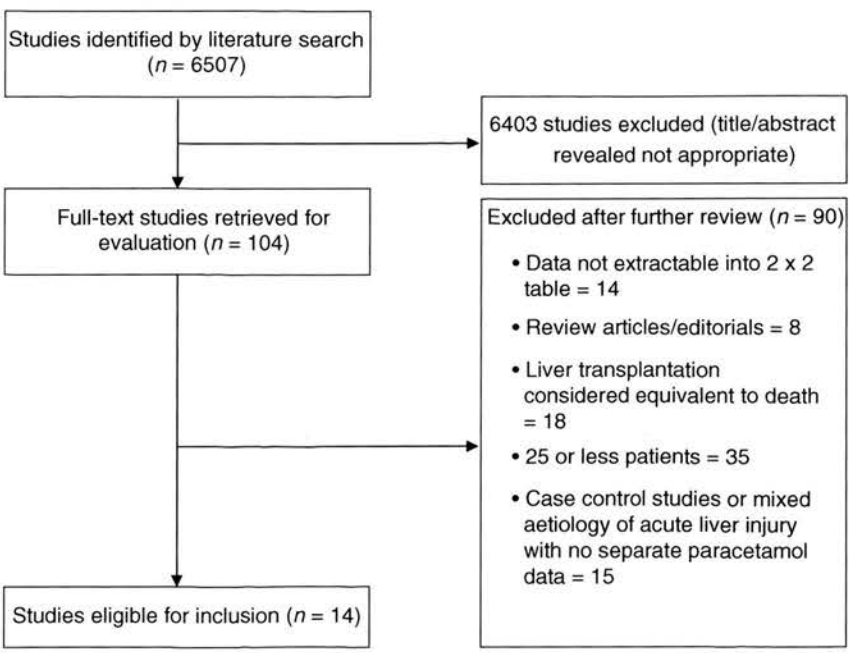

Figure 1. Flow diagram of assessment of studies identified in the systematic review.

Table 3. Characteristics of included studies

Study	Country	Liver unit	Study period	Inclusion criteria	Prognostic test(s) evaluated	No paracetamol patients included	Retrospective/ prospective cohort	Retrospective/ prospective test development/ evaluation	Study quality
O'Grady *et al.*[5]	UK	KCH	1973–1985; 1986–1987	FHF as per Trey and Davidson[50] with grade III–IV HE	pH <7.3 Concurrent PT >100 s, serum creatinine >300 mmol/L, grade III/IV HE	121 99	Both	Prospective	Moderate
O'Grady *et al.*[23]	UK	KCH	1988–1990	Severe liver damage	KCC as per O'Grady *et al.*[5]	60	Prospective	Prospective	Moderate
Izumi *et al.*[24]	UK	KCH	Not stated	FHF as per Trey and Davidson[50]	KCC as per O'Grady *et al.*[5] Factor V ratio <20% Factor V ratio <10%	81	Prospective	Prospective (partially)	Poor
Anand *et al.*[25]	UK	Birmingham	1990–1994	FHF as per Trey and Davidson[50]	pH <7.3 Concurrent PT >100 s, serum creatinine >300 mmol/L, grade III/IV HE	72 89	Retrospective	Prospective	Poor
Bernal *et al.*[26]	UK	KCH	1990–1996	Severe hepatotoxicity (KCC); Death whilst not meeting KCC (APACHE III)	KCC as per O'Grady *et al.*[5] Adapted APACHE III	504 56	Retrospective	Prospective (KCC) Retrospective (APACHE III)	Poor
Mitchell *et al.*[27]	UK	KCH	1993–1994	Coagulopathy + recent history paracetamol ingestion	KCC as per O'Grady *et al.*[5] APACHE II >15 at 24 hr post-admission APACHE II >15	94	Prospective	Prospective (KCC) Retrospective (APACHE II)	Good
Bernal *et al.*[7]	UK	KCH	1998–1999 (learning set) 1999–2000 (validation set)	Severe paracetamol-induced hepatotoxicity	KCC as per O'Grady *et al.*[5] Arterial lactate >3.5 mmol/L at 4 h Arterial lactate >3.0 mmol/L at 12 h KCC + combination of lactate criteria	99 97 85 85	Both	Prospective	Moderate

Table 3. (Continued)

Study	Country	Liver unit	Study period	Inclusion criteria	Prognostic test(s) evaluated	No paracetamol patients included	Retrospective/ prospective cohort	Retrospective/ prospective test development/ evaluation	Study quality
Bernal and Wendon[28]	UK	KCH	1998–2000	Acute severe hepatotoxicity	KCC as per O'Grady *et al.*[5] Phosphate >1.2 mmol/L on admission day +1 or 2	170	Retrospective	Prospective	Moderate
Larson *et al.*[29]	USA	22 academic centres	1998–2003	INR >1.4; HE; jaundice to HE interval <26 weeks	KCC as per O'Grady *et al.*[5]	252	Prospective	Prospective	Moderate
Schmidt and Dalhoff[10]	Denmark	Copenhagen	1999–2002	Peak ALT >1000 U/L	KCC as per O'Grady *et al.*[5]	234	Prospective	Prospective (KCC) Retrospective (AFP)	Moderate
					AFP <3.9 at D1 postpeak ALT	188			
					AFP <3.9 and INR >2.4 at D1 postpeak ALT	188			
Schmidt and Larsen[30]	Denmark	Copenhagen	1999–2004	Severe paracetamol-induced FHF	KCC as per O'Grady *et al.*[5]	95	Prospective	Retrospective	Moderate
					Arterial lactate	91			
					Modified KCC	91			
					SOFA score >8 at admission/>12 at onset of grade III/IV HE	95			
					SIRS at admission/at onset of grade III/IV HE	95			
Zaman *et al.*[31]	Ireland	Dublin	1994–2005	Paracetamol-induced ALF (jaundice to HE <8 weeks) or rapid ↑ bilirubin/INR/ renal impairment/ hypoglycaemia if no HE	KCC as per O'Grady *et al.*[5] MELD >30	60	Retrospective	Prospective	Poor

Table 3. (Continued)

Study	Country	Liver unit	Study period	Inclusion criteria	Prognostic test(s) evaluated	No paracetamol patients included	Retrospective/ prospective cohort	Retrospective/ prospective test development/ evaluation	Study quality
Bates *et al.*[32]	UK	Edinburgh	2004–2007	Acute severe liver injury	KCC as per O'Grady *et al.*[5]	69	Retrospective	Prospective	Poor
					Lactate modifications to KCC as per Bernal *et al.*[7]	32			
Bernal *et al.*[33]	UK	KCH	Not stated	Acute liver failure	IL-6 within 24 h of admission	31	Prospective	Retrospective	Poor

PT, prothrombin time; INR, International Normalised Ratio; FHF, fulminant hepatic failure; APACHE II, Acute Physiology and Chronic Health Evaluation II; ALT, alanine aminotransferase; AFP, alpha-fetoprotein; KCC, King's College Criteria; SOFA, Sequential Organ Failure Assessment, MELD, Model for End-Stage Liver Disease; KCH, King's College Hospital.

total of 1929 patients. One study[29] evaluated the KCC only on admission. After exclusion of studies with complete temporal overlap with other studies,[10, 24, 27] pooled specificity of the KCC was high, at 94.6% (95% CI 93.0–95.9), but the pooled sensitivity was relatively poor at 58.2% (95% CI 53.1–63.3). The pooled DOR for the KCC was 27.7 (95% CI 9.2–83.5; Table 4). The summary ROC curve is shown in Figure 2. The area under the curve (*AUC*) was calculated as 0.91 (95% CI 0.79–0.99), suggesting good performance of the KCC overall.[34] The accuracy of the KCC improved when studies originating from King's College Hospital (KCH) were analysed separately from those outside KCH [DOR 43.9 (95% CI 17.6–109.3) for KCH-based studies vs. DOR 16.5 (95% CI 3.5–77.8) for non-KCH-based studies]. Statistically significant heterogeneity (I^2 = 87%) existed between individual study results, although this fell to 79% after exclusion of the single multicentre study,[29] and to 64% after exclusion of studies from outside KCH.

Lactate modifications to the KCC

The original study[7] that evaluated arterial lactate in the prognosis of paracetamol-induced ALF reported similar specificity, but improved sensitivity, when compared with the original KCC. However, two[30, 32] subsequent studies evaluating arterial lactate alone failed to replicate this high prognostic accuracy (Table 4), with pooled DORs of 12.2 (95% CI 4.0–37.4) and 22.8 (95% CI 2.5–210.0) for the early and postresuscitation lactate values respectively. This was mainly because of the reduced specificity seen in the two studies from outside KCH. Combination of the KCC with a postresuscitation lactate value >3.0 offered a higher prognostic accuracy in the original lactate study,[7] but this was not replicated in a subsequent evaluation.[30]

Other prognostic markers

Several other markers, all evaluated in single studies, appeared to offer improved prognostic accuracy when compared with the KCC, with low-serum alpha-fetoprotein (AFP) (DOR 419.1),[10] 24-h post-admission Acute Physiology and Chronic Health Evaluation II (APACHE II) score (DOR 143.0),[27] serum interleukin-6 (IL-6) levels (DOR 66.0),[33] Model for End-Stage Liver Disease (MELD) score (DOR 58.8),[31] and serum phosphate (DOR 33.9)[28] all outperforming the KCC

Table 4. Sensitivity, specificity and diagnostic odds ratios of individual prognostic markers

Prognostic test	Study	*N*/deaths	Test +ve/ deaths	Sensitivity (95% CIs)	Specificity (95% CIs)	Diagnostic odds ratio (95% CIs)	Heterogeneity χ^2 (*P*-value)	Heterogeneity I^2 (%)
pH <7.3	O'Grady *et al.*[5]	121/43	22/21	48.8 (33.3–65.5)	98.7 (93.1–100)	73.5 (9.4–577.4)	4.91 (0.027)	80
	Anand *et al.*[25]	72/39	31/24	61.5 (44.6–76.6)	78.8 (61.1–91.0)	5.9 (2.1–17.1)		
	Pooled			54.9 (43.5–65.9)	92.8 (86.3–96.8)	18.0 (1.1–229.6)		
Concurrent PT >100 s, serum creatinine >300 mmol/L, grade III/IV HE	O'Grady *et al.*[5]	99/22	15/10	45.5 (24.4–67.8)	93.5 (85.5–97.9)	12.0 (3.5–41.3)	0.8 (0.374)	0
	Anand *et al.*[25]	89/45	24/19	42.2 (27.7–57.9)	88.6 (75.4–96.2)	5.7 (1.9–17.2)		
	Pooled			43.3 (31.2–56.0)	91.7 (85.3–96.0)	7.9 (3.5–18.1)		
KCC (combined)*	O'Grady *et al.*[5]	220/65	37/31	47.7 (35.1–60.5)	96.1 (91.8–98.6)	22.6 (8.8–58.6)	8.4 (0.038)	64
	O'Grady *et al.*[23]	60/26	23/19	73.1 (52.2–88.4)	88.2 (72.6–96.7)	20.4 (5.2–79.0)		
	Bernal *et al.*[26]	504/99	80/71	71.7 (61.8–80.3)	97.8 (95.8–99.0)	111.6 (50.5–246.4)		
	Bernal *et al.*[7]	99/21	20/16	76.2 (52.8–91.8)	94.9 (87.4–98.6)	59.2 (14.3–245.3)		
	Pooled (KCH)			65.9 (59.0–72.3)	96.1 (94.4–97.5)	43.9 (17.6–109.3)		
	Larson *et al.*[29] (at admission)	252/74	34/19	25.7 (16.2–37.2)	91.6 (86.5–95.2)	3.8 (1.8–7.9)	17.0 (0.001)	82
							53.0 (<0.001)	87
	Schmidt and Larsen[30]	95/48	36/27	77.1 (62.7–88.0)	83.0 (69.2–92.4)	16.4 (5.9–45.3)		
	Zaman *et al.*[31]	60/29	21/21	72.4 (52.8–87.3)	100.0 (88.8–100.0)	159.4 (8.7–2908.9)		
	Bates *et al.*[32]	69/17	18/15	88.2 (63.6–98.5)	94.2 (84.1–98.8)	122.5 (18.7–803.1)		
	Pooled (non-KCH)			48.8 (41.0–56.6)	91.2 (87.5–94.1)	16.5 (3.5–77.8)		
	Pooled (overall)			58.2 (53.1–63.3)	94.6 (93.0–95.9)	27.7 (9.2–83.5)		
Arterial lactate >3.5 at admission	Bernal *et al.*[7]	97/21	18/14	66.7 (43.0–85.4)	94.7 (87.1–98.6)	36.0 (9.3–139.6)	4.54 (0.103)	56
	Schmidt and Larsen[30]	91/46	61/39	84.8 (71.1–93.7)	51.1 (35.8–66.3)	5.8 (2.2–15.8)		
	Bates *et al.*[32]	69/17	37/15	88.2 (63.6–98.5)	57.7 (43.2–71.3)	10.2 (2.1–49.4)		
	Pooled			81.0 (70.9–88.7)	72.3 (64.9–78.8)	12.2 (4.0–37.4)		
Arterial lactate >3.0 following resuscitation	Bernal *et al.*[7]	85/21	18/16	76.2 (52.8–91.8)	96.9 (89.2–99.6)	99.2 (17.6–559.3)	9.83 (0.007)	80
	Schmidt and Larsen*[30]	91/46	55/36	78.3 (63.6–89.1)	57.8 (42.2–72.3)	4.9 (2.0–12.3)		
	Bates *et al.*[32] *at onset of grade III/IV HE	32/12	20/12	100.0 (73.5–100.0)	60.0 (36.1–80.9)	36.8 (1.9–708.0)		
	Pooled			81.0 (70.6–89.0)	77.5 (69.3–84.4)	22.8 (2.5–210.0)		
Arterial lactate >4.0 at admission	Schmidt and Larsen[30]	91/46	51/34	73.9 (58.9–85.7)	62.2 (46.5–76.2)	4.7 (1.9–11.4)	–	–
Arterial lactate >4.0 at onset of grade III/IV HE			40/31	67.4 (52.0–80.5)	80.0 (65.4–90.4)	8.3 (3.2–21.5)		
KCC + arterial lactate >3.0 following resuscitation	Bernal *et al.*[7]	85/21	24/19	90.5 (69.6–98.8)	92.2 (82.7–97.4)	112.1 (20.1–625.7)	8.83 (0.003)	89
	Schmidt and Larsen[30]	91/46	69/42	91.3 (79.2–97.6)	40.0 (25.7–55.7)	7.0 (2.1–22.9)		
	Pooled			91.0 (81.5–96.6)	70.6 (61.2–79.0)	26.1 (1.7–393.7)		
Factor V ratio <20%	Izumi *et al.*[24]	81/35	69/34	97.1 (85.1–99.9)	23.9 (12.6–38.8)	10.7 (1.3–87.3)	–	–

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Table 4. (Continued)

Prognostic test	Study	*N*/deaths	Test +ve/ deaths	Sensitivity (95% CIs)	Specificity (95% CIs)	Diagnostic odds ratio (95% CIs)	Heterogeneity χ^2 (*P*-value)	Heterogeneity I^2 (%)
Factor V ratio <10%	51/29	63.0 (47.6–76.8)	37.1 (21.5–55.1)	5.3 (1.8–15.1)				
Adapted APACHE III	Bernal *et al.*[26]	56/28	19/16	57.1 (37.2–75.5)	89.3 (71.8–97.7)	11.1 (2.7–45.6)	–	–
APACHE II >15 at 24 hr post-admission	Mitchell *et al.*[27]	94/14	13/11	78.6 (49.2–95.3)	97.5 (91.3–99.7)	143.0 (21.4 –953.5)	–	–
APACHE II >15 within 5 days of admission			23/13	92.9 (66.1–99.8)	87.5 (78.2–93.8)	91.0 (10.7–772.8)		
Phosphate >1.2 mmol/L on admission day +1 or 2	Bernal and Wendon[28]	170/52	55/42	80.8 (67.5–90.4)	89.0 (81.9–94.0)	33.9 (13.8–83.3)	–	–
AFP <3.9 at D1 postpeak ALT	Schmidt and Dalhoff[10]	188/33	54/33	100.0 (89.4–100.0)	73.6 (65.9–80.3)	184.9 (11.1–3085.6)	–	–
AFP <3.9 and INR >2.4 at D1 postpeak ALT			74/33	100.0 (89.4–100.0)	86.5 (80.0–91.4)	419.1 (24.8–7097.5)		
SOFA score >8 at admission	Schmidt and Larsen[30]	95/48	48/32	66.7 (51.6–79.6)	66.0 (50.7–79.1)	3.9 (1.7–9.1)	–	–
SOFA score >12 at onset of grade III/IV HE			54/39	81.3 (67.4–91.1)	68.1 (52.9–80.9)	9.2 (3.6–23.9)		
SIRS at admission	Schmidt and Larsen[30]	95/48	52/33	68.8 (53.7–81.3)	59.6 (44.3–73.6)	3.2 (1.4–7.5)	–	–
SIRS at onset of grade III/IV HE			41/34	70.8 (55.9–83.0)	85.1 (71.7–93.8)	13.9 (5.0–38.3)		
MELD >30	Zaman *et al.*[31]	60/29	38/28	96.6 (82.2–99.9)	67.7 (48.6–83.3)	58.8 (7.0–495.8)	–	–
IL-6 within 24 h of admission	Bernal *et al.*[33]	31/8	6-Jul	75.0 (34.9–96.8)	95.7 (78.1–99.9)	66.0 (5.1–857.7)	–	–

PT, prothrombin time; APACHE II, Acute Physiology and Chronic Health Evaluation II; ALT, alanine aminotransferase; AFP, alpha-fetoprotein; KCC, King's College Criteria; SOFA, Sequential Organ Failure Assessment, MELD, Model for End-Stage Liver Disease; KCH, King's College Hospital.

* Anand *et al.*[25] excluded as 2 × 2 table not reconstructable; Mitchell *et al.*,[27] Bernal and Wendon[28] and Schmidt and Dalhoff[10] excluded because of complete temporal overlap with previous studies from the same unit.

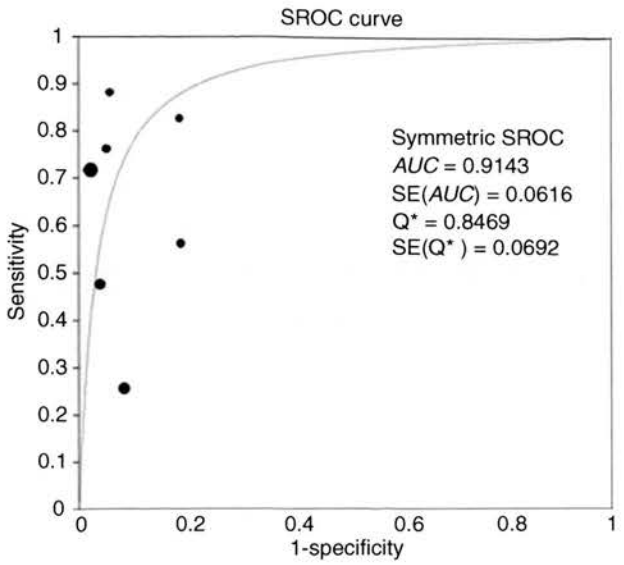

Figure 2. Summary receiver operator curve (SROC) of studies evaluating the original KCC. *AUC*, area under the curve; S.E., standard error; *Q*, heterogeneity.

(Table 4). However, further studies would be required to replicate these findings to confirm or refute whether these other markers are indeed superior to the KCC.

DISCUSSION

This systematic review and meta-analysis has demonstrated that the original KCC for paracetamol-induced ALF have high pooled specificity (94.6%), but low pooled sensitivity (58.2%) in determining prognosis in patients with paracetamol-induced ALF. Additionally, the benefit of the arterial lactate modifications to the KCC[7] is questionable according to these data. Other proposed prognostic markers, in particular AFP, APACHE II scores and serum IL-6 levels, showed encouraging prognostic accuracy, but were only evaluated in single studies of variable quality.

This review is limited by the quality of the included studies, which had significant heterogeneity and were generally of poor or moderate quality, with only two[5, 7] studies validating their prognostic model prospectively in a separate cohort. ALF is a rare syndrome and, as a result, many studies were small and retrospective in nature. Continuous variables were frequently analysed retrospectively using cut-off values designed to maximize *AUC* values, a method which assumes constant risk amongst the 'high' and 'low' risk groups.[18] Several studies attempted to evaluate multiple prognostic markers retrospectively using the same cohort, an approach which increases the risk of obtaining a statistically significant result by chance. Another potentially confounding issue is the lack of international standardization of laboratory variables, such as prothrombin time and creatinine, and changes to assay reagents over the time course of these studies.[35] We also appreciate that exclusion of studies where transplantation and death were deemed equivalent may have introduced spectrum bias, as prognostic tests are usually applied in settings where OLT is available. This problem is difficult to circumvent given that emergency OLT will never be subjected to a clinical trial, but, given the reduced quality-of-life seen following transplantation for paracetamol-induced ALF, the accumulated risks of immunosuppression and the scarcity of liver donors,[16] accurate calculation of the specificity of each prognostic test is vital in order to minimize inappropriate transplantation.

This study expands upon a recent systematic review[16] evaluating the KCC for paracetamol-induced ALF by including additional prognostic markers. The former study found spectrum bias in studies originating from KCH, with increased spontaneous survival amongst patients listed for transplantation, but not receiving a graft, compared with those meeting criteria, but never listed, suggesting that a 'healthier' cohort may be preferentially transplanted in KCH. The current data confirm the increased prognostic accuracy of the original KCC in studies originating from KCH compared with studies from other units, with a higher pooled DOR in KCH-based studies compared with that from studies conducted in other units. This raises further questions regarding the overall generalizability of the KCC to patients with paracetamol-induced ALF treated outside KCH.

The heterogeneity of the included studies evaluating the KCC was partly because of a multicentre US study[29] which explicitly applied the KCC solely at the time of admission, rather than dynamically throughout admission as originally intended.[5, 36] Whilst early and accurate prognostication in ALF is vital to permit timely listing for OLT, application of the KCC solely at admission reduces the time available for the disease to evolve and may introduce spectrum bias. Given that the median time taken to fulfil the KCC following admission to a tertiary liver transplant unit is 12 h,[7] application of the KCC solely at admission may

explain the low sensitivity of the criteria in the US study, whilst concerns have also been expressed about the prophylactic use of fresh frozen plasma in this US study and the potential confounding effects upon prothrombin time.[37] Furthermore, 48% of cases resulted from unintentional overdoses in this US study, a type of overdose seen less frequently in the UK.[38] It may be that repeated ingestion of supratherapeutic doses of paracetamol over a protracted time course disrupts liver function subacutely, so that patients are less likely to develop the profound acidosis or concurrent severe coagulopathy, renal dysfunction, and encephalopathy required to fulfil the KCC, but have at least as poor a prognosis as an intentional overdose at a single time point. Future prognostic scoring systems may therefore need to take the pattern of paracetamol overdose into account in addition to traditional biochemical parameters.

The addition of postresuscitation arterial lactate to the KCC[7] is questioned by this study. Only two additional[30, 32] studies, one of which was reported in abstract-form, that reported this modification fulfilled eligibility criteria for inclusion, but these studies suggest little benefit from this. Furthermore, the reduced specificity of the lactate criteria undermines the traditional benefit of the KCC in 'ruling in' a hopeless prognosis. The presence of systemic inflammation, with or without sepsis, is increasingly recognized as important in ALF,[39] but hyperlactataemia can result from numerous other organ sources and therefore perhaps not surprisingly, arterial lactate is a relatively nonspecific prognostic indicator in paracetamol-induced ALF. Within critical care settings, the use of APACHE II and Sequential Organ Failure Assessment (SOFA) scores to monitor organ dysfunction have greater recognition than the KCC and are attractive as early prognostic markers in ALF.[40] APACHE II, in particular, showed encouraging prognostic accuracy, but was only evaluated in a single eligible study.[27] One[29] additional study evaluating APACHE II scores was excluded from this particular analysis, as the prognostic scoring reported in the study could not be reconstructed into a 2×2 table; this study is also notable for the atypical nature of the patient cohort as outlined above. Given that APACHE III, systemic inflammatory response syndrome (SIRS) and SOFA scores all performed less well than the KCC (albeit in single studies), the role of these markers may be to permit earlier identification of a high-risk cohort requiring transfer to tertiary centres that offer liver transplantation, rather than as definitive transplant listing criteria.

Persistently elevated serum phosphate or reduced AFP levels may reflect failure of hepatic regeneration and therefore could help predict a poorer outcome in paracetamol-induced ALF. Serum AFP showed high prognostic performance in one[10] study, whereas serum phosphate showed equivalence with the KCC in one[28] retrospective study from KCH. Other authors (in studies where transplantation was equated with death and hence excluded from this study) have demonstrated conflicting results with serum phosphate[41–44] and therefore further evaluation of both serum AFP and phosphate in future studies would be worthwhile. The MELD scoring system has been widely adopted for organ allocation in chronic liver disease and has shown encouraging prognostic accuracy in nonparacetamol ALF.[12, 45–47] More limited data exist regarding the use of MELD in paracetamol-induced ALF, but one additional study (excluded from this analysis as the 2×2 table was not reconstructable) suggested that its use may be limited by a high false positive rate.[48]

In summary, this systematic review and meta-analysis has demonstrated that the original KCC for paracetamol-induced ALF have high pooled specificity, but low pooled sensitivity in determining prognosis in patients with paracetamol-induced ALF. The KCC had reduced prognostic accuracy when applied outside KCH and were occasionally applied only at admission. The reduced specificity of the KCC following the addition of arterial lactate calls into question the benefit of this modification, suggesting that re-evaluation of this as a prognostic marker is required. Urgent consideration should be given to the design of a high-quality, prospective study evaluating the KCC, APACHE II scores and markers of hepatic regeneration, such as serum AFP and phosphate, in paracetamol-induced ALF. Given the relatively rare nature of ALF, such a study is likely to require cooperation between several large centres and argues the need for a collaborative network of tertiary hospitals experienced in the management and prognostication of ALF, similar to that developed by the Acute Respiratory Distress Syndrome Network program.[49]

ACKNOWLEDGEMENT

Declaration of personal and funding interests: None.

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BJCP British Journal of Clinical Pharmacology

DOI:10.1111/j.1365-2125.2010.03819.x

Overdose pattern and outcome in paracetamol-induced acute severe hepatotoxicity

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Keywords
acute, drug-induced liver injury, hepatic encephalopathy, liver failure, liver transplantation

Received
18 May 2010
Accepted
31 August 2010

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Paracetamol hepatotoxicity is the commonest cause of acute liver failure (ALF) in the UK.
- Conflicting data exist regarding the impact of overdose pattern upon subsequent mortality or need for emergency liver transplantation.

WHAT THIS STUDY ADDS

- Unintentional paracetamol overdose is independently associated with reduced survival compared with intentional overdose.
- Unintentional paracetamol overdoses should be treated as high-risk for the development of multiorgan failure, and should be considered for *N*-acetyl cysteine treatment irrespective of admission serum paracetamol concentrations.
- The King's College poor prognostic criteria have reduced sensitivity in unintentional overdose patients and alternative prognostic criteria may be required.

AIMS

Paracetamol (acetaminophen) hepatotoxicity is the commonest cause of acute liver failure (ALF) in the UK. Conflicting data regarding the outcomes of paracetamol-induced ALF resulting from different overdose patterns are reported.

METHODS

Using prospectively defined criteria, we have analysed the impact of overdose pattern upon outcome in a cohort of 938 acute severe liver injury patients admitted to the Scottish Liver Transplantation Unit.

RESULTS

Between 1992 and 2008, 663 patients were admitted with paracetamol-induced acute severe liver injury. Of these patients, 500 (75.4%) had taken an intentional paracetamol overdose, whilst 110 (16.6%) had taken an unintentional overdose. No clear overdose pattern could be determined in 53 (8.0%). Unintentional overdose patients were significantly older, more likely to abuse alcohol, and more commonly overdosed on compound narcotic/paracetamol analgesics compared with intentional overdose patients. Unintentional overdoses had significantly lower admission paracetamol and alanine aminotransferase concentrations compared with intentional overdoses. However, unintentional overdoses had greater organ dysfunction at admission, and subsequently higher mortality (unintentional 42/110 (38.2%), intentional 128/500 (25.6%), $P < 0.001$). The King's College poor prognostic criteria had reduced sensitivity in unintentional overdoses (77.8%, 95% confidence intervals (CI) 62.9, 88.8) compared with intentional overdoses (89.9%, 95% CI 83.4, 94.5). Unintentional overdose was independently predictive of death or liver transplantation on multivariate analysis (odds ratio 1.91 (95% CI 1.07, 3.43), $P = 0.032$).

CONCLUSIONS

Unintentional paracetamol overdose is associated with increased mortality compared with intentional paracetamol overdose, despite lower admission paracetamol concentrations. Alternative prognostic criteria may be required for unintentional paracetamol overdoses.

Introduction

Acute liver failure (ALF) occurs following sudden extensive loss of liver cell mass, resulting in hepatic encephalopathy (HE) and coagulopathy, and can lead to multiple organ failure with a high associated mortality rate. Previous studies have highlighted the major contribution of paracetamol (acetaminophen) as a cause of ALF in the UK, North America and Europe [1–3]. However, there are significant differences in the epidemiology of paracetamol-induced ALF in North America compared with the UK. Data from the USA, covering 1990–99, suggested paracetamol overdose was responsible for 56 000 emergency department visits, 26 000 hospital admissions and 458 deaths each year [4]. Approximately 12 650 (22.6%) emergency department visits, 2240 (8.6%) admissions and 100 (21.8%) deaths were due to unintentional paracetamol ingestion [4]. Against this background the US Acute Liver Failure Study group reported that unintentional overdose was the most common pattern of ingestion in patients with ALF, responsible for 48% of all overdoses, and reported similar outcomes between intentional and unintentional overdoses [2]. These data contrast with the pattern of overdose reported in the UK. In the King's College Hospital series, 92% of paracetamol-induced ALF occurred after ingestion at a single time point with suicidal intent [5]. Contradictory data have also been presented regarding the outcome of ALF induced by accidental (unintentional) overdose of paracetamol, with increased mortality [6], or similar outcomes [2, 7], being reported. Lastly, in those series reporting increased mortality in patients following accidental paracetamol poisoning, it is unclear if this excess mortality is associated with this pattern of overdose *per se* or other clinical features such as organ failure or alcohol consumption prevalent in this population [6].

The aim of this cohort study was to analyse the incidence and outcome of unintentional paracetamol overdoses compared with intentional overdoses utilizing prospectively defined data collected from 938 patients with acute severe liver injury admitted to the Scottish Liver Transplantation Unit (SLTU).

Methods

Patients

The cohort retrospectively analysed was from 938 patients admitted to the SLTU between 1 November 1992 and 31 October 2008 with suspected severe acute liver injury. Severe acute liver injury was defined as sudden deterioration in liver function with associated coagulopathy in the absence of a history of chronic liver disease, whilst the term ALF (i.e. fulminant liver failure) was restricted to those patients developing hepatic encephalopathy (HE) [8]. Guidelines for accepting patients from referring hospitals were based on previously published criteria and have remained unchanged over the time course of the study [9]. These admission criteria included: HE, progressive coagulopathy with a prothrombin time (PT) > 50 s, international normalized ratio (INR) > 5 or in the case of paracetamol overdose, PT in seconds greater than time in hours post overdose, persistent metabolic acidosis despite adequate fluid resuscitation, hypoglycaemia or deteriorating renal function in the presence of severe liver injury. Following admission a detailed clinical history, examination and laboratory investigations were performed, with imaging studies and transjugular liver biopsy undertaken where clinically indicated. Laboratory investigations were repeated at daily intervals or more frequently in patients with rapidly progressive liver failure. Patients admitted to the SLTU are managed using a standard protocol as previously described, which is reviewed on an annual basis [10]. The King's College Hospital poor prognostic criteria (KCC) are used in this unit and throughout the UK to determine patients who will most likely die without liver transplantation (LT) [11]. The KCC were modified in 2006 within the UK to include arterial lactate concentration in patients with paracetamol overdose [12]. Liver transplantation (LT) was considered in all patients meeting KCC in conjunction with their medical condition and psychological assessment. If accepted as transplant candidates, patients are 'super-urgently' listed with UK Transplant and prioritized for the next available compatible organ.

Methods

Details of patient history, clinical examination and laboratory results along with therapeutic interventions, including intensive care admission, need for renal replacement therapy or inotropic support, were prospectively recorded in the ALF database. The following variables were recorded at the time of admission: temperature, pulse, white cell count (WCC), platelet count, INR, serum electrolytes, serum bilirubin, alanine aminotransferase (ALT), serum albumin, arterial hydrogen ion, bicarbonate and arterial lactate. Where available, the paracetamol preparation, number of tablets, type (whether accidental or intentional) and timing of overdose, delay to presentation and use of *N*-acetylcysteine (NAC) were all recorded. Background information such as alcohol use and dependency, illicit drug use, pre-existing psychiatric history and employment was obtained by the admitting medical team from a variety of sources including, where possible, the patient, the patient's family and the patient's general medical practitioner. The suicidal ideation of each patient was assessed by detailed interview of the patient (when the absence of HE permitted this) by the specialist transplant psychiatric liaison team prior to any decisions regarding listing for LT, with corroborating evidence obtained from the patient's family and general practitioner where possible. Where

available, further information was obtained from review of the medical and psychiatric notes from the referring hospital.

Definitions

Paracetamol overdose was prospectively assigned as the cause of acute severe liver injury if there was a clear history of ingestion of potentially toxic amounts of paracetamol (>4 g day^{-1}) within 7 days of presentation, serum paracetamol concentrations were >10 mg l^{-1} or serum ALT concentration was >1000 IU l^{-1} within 7 days of a history of paracetamol ingestion irrespective of the serum paracetamol concentration [2]. Paracetamol overdose was only accepted as the cause of acute severe liver injury after exclusion of other potential aetiologies, in particular the presence of other hepatotoxic drugs or substances, hepatitis A and B, autoimmune hepatitis and Wilson's disease.

An *intentional overdose* was defined as a cumulative dose of paracetamol >4 g ingested over 4 h or less with the objective of self-harm; *unintentional overdose* was defined as a paracetamol overdose ingested when self-harm was not the aim. *Single overdose* was an overdose (>4 g) taken at a single defined time point whilst a *staggered overdose* described ingestion of two or more supratherapeutic paracetamol doses over a time interval of greater than 8 h resulting in a cumulative dose of >4 g day^{-1}. *Mixed overdose* described more than one type of tablet being taken at or during the time of the overdose, whilst *compound overdose* described overdose of compound tablets which included paracetamol such as co-proxamol or co-dydramol. *Alcohol abuse* was defined as active and resistant alcohol dependence in excess of 56 units week^{-1} for men and 42 units week^{-1} for women.

Outcome was defined as spontaneous survival to discharge without transplant, death without transplant, survival with transplant and death with transplant. When undertaking survival analysis, death and LT were considered equivalent.

Statistical analysis

All patient data were prospectively recorded in the SLTU ALF database. Statistical analysis was retrospectively performed using SPSS software (SPSS 16.0, Chicago IL, USA) and Graphpad Prism (GraphPad Software Inc., La Jolla, CA). Data values are presented as median and interquartile range (IQR) or percentages unless otherwise stated. Continuous data were compared using analysis of variance or the Kruskal-Wallis test for non-normally distributed variables. Categorical data were analysed using Chi-squared tests or Fisher's exact test. The Bonferroni method was used to adjust P values to account for multiple comparisons. Stepwise logistic regression was used to determine factors predictive of death or LT in paracetamol-induced acute severe liver injury patients. Only variables with $P < 0.10$ were included in the multivariate analysis. Actuarial probability curves were constructed using the Kaplan-Meier method and compared with log-rank testing. A two-sided P value of less than 0.05 was considered statistically significant.

Results

Overall study population

Over a 16 year period (12 November 1992–11 November 2008) 938 patients were admitted to the SLTU with suspected severe acute liver injury, of whom 663 (70.7%) were prospectively classified as having paracetamol-induced hepatotoxicity. Six hundred and fourteen (92.6%) had a history of potentially toxic paracetamol consumption (>4 g day^{-1}). Six hundred and twenty-eight patients (94.7%) had an ALT >1000 IU l^{-1} [ALT >3500 in 526 (79.3%)] and 512 (77.2%) had detectable paracetamol in serum. Only four of these 663 patients (0.6%) fulfilled only one criterion for paracetamol-induced liver injury. All four of these patients had a history of ingestion of potentially toxic quantities of paracetamol but serum paracetamol was undetectable and ALT < 1000 IU l^{-1}. Two of these patients had a clear history of a single ingestion of a large quantity of paracetamol with suicidal intent and two had a history of staggered unintentional paracetamol ingestion. The latter two became encephalopathic, one fulfilled KCC and both died without transplant. The two former patients survived without LT. These four patients have been included in subsequent analysis as paracetamol cases. Baseline demographic and clinical characteristics of the paracetamol study group are outlined in Table 1.

Clinical presentation

Of the 663 paracetamol cases, 520 (78.4%) had been transferred to the SLTU from a total of 14 separate health authorities, with the remaining 143 patients transferred from local hospitals or from wards within the Royal Infirmary of Edinburgh. In those patients (414/663, 62.4%) in whom accurate timings could be obtained, presentation to emergency services occurred at a median of 23 h post final paracetamol ingestion (range 1–130 h). Female admissions accounted for 348 (52.5%) of admissions, with 315 (47.5%) males admitted. The median age at admission was 35 (IQR 27–45) years for males and 34 (IQR 24–44) years for females. Information regarding NAC use in the referring hospital was available for 644/663 (97.1%) of patients, of whom 559 (86.8%) had received intravenous NAC, at a median time from last paracetamol ingestion of 23.75 (IQR 10–44) h. A total of 263/581 (45.3%) patients had a history of chronic alcohol abuse, with 264/590 (44.7%) of patients taking alcohol concomitantly with their overdose. In those patients (n = 606) in whom an employment history could be obtained, 289 (47.7%) patients were unemployed, 220 (36.3%) were employed, and 27 (4.5%) were in full time education. A total of 244/577 (42.3%) patients had a prior history of psychiatric illness and 242/619 (39.1%) had

Table 1

Admission characteristics of 663 subjects with paracetamol-induced acute severe liver injury

Admission characteristic (n = 663 unless otherwise stated)		
Sex (male/female)		315/348 (47.5/52.5%)
Age (years)		34 (26–44)
Pattern of overdose		
Intentional		500 (75.4%)
Accidental		110 (16.6%)
Unknown		53 (8.0%)
Time course of overdose		
Single		450 (67.9%)
Staggered		161 (24.3%)
Unknown		52 (7.8%)
Paracetamol concentration (mg l^{-1}) (n = 561)		60.5 (20–130)
Mixed overdose (n = 620)		316 (51.0%)
Associated alcohol with overdose (n = 590)		264 (44.7%)
Alcohol abuse† (n = 581)		263 (45.3%)
Previous psychiatric history (n = 577)		244 (42.3%)
Active drug abuse (n = 623)		96 (15.4%)
Previous overdose (n = 619)		242 (39.1%)
Unemployed at time of overdose (n = 606)		289 (47.7%)
Time from overdose to SLTU admission (h) (n = 414)		52 (40–67)
Received NAC in referring hospital (n = 644)		559 (86.8%)
Admission laboratory parameters	WCC (x10^9 l^{-1})	10.7 (7.6–14.5)
	Platelets (x10^9 l^{-1})	125 (73–174)
	Creatinine (μmol l^{-1})	134 (84–234)
	ALT (IU l^{-1})	7 291 (4 250–10 130)
	Bilirubin (μmol l^{-1})	83 (58–113)
	Albumin (g l^{-1})	35 (31–39)
	PT (s)	48 (34–67)
Ever encephalopathic		344 (51.9%)
Never encephalopathic		319 (48.1%)
Not encephalopathic on admission		362 (54.6%)
Developed encephalopathy during admission (n = 362)		43 (11.9%)
Overall outcome		
Survived without transplant		446 (67.3%)
Died without transplant		165 (24.9%)
Survived with transplantation (to hospital discharge)		37 (5.6%)
Died with transplantation		15 (2.2%)

Data are presented as median (IQR) or numbers (%) as appropriate. †>56 units $week^{-1}$ (male); >42 units $week^{-1}$ (female). Abbreviations: WCC, white cell count; ALT, alanine aminotransferase; PT, prothrombin time.

taken a previous overdose. A total of 96/623 (15.4%) patients admitted to current recreational drug use. A total of 301 (45.4%) of paracetamol-induced acute liver injury patients were encephalopathic on admission, and a further 43/362 (11.9%) went on to develop HE during admission. A total of 344 (51.9%) of patients therefore developed HE, and thus ALF, at some point during their illness.

Intentional vs. unintentional overdoses

Of the 610 (92%) patients in whom a clear psychiatric history could be obtained, 500/610 (82%) reported an intentional (suicidal) overdose, whilst 110/610 (18%) subjects denied suicidal ideation and were classified as unintentional overdoses (Table 2). Unintentional overdose subjects were significantly older (median 40 (IQR 30–48) years) compared with intentional overdose patients (33 (24–43) years, $P < 0.001$), had a lower admission paracetamol concentration (34.7 (15.9–57.5) mg l^{-1} *vs.* 75.6 (25.4–148.2) mg l^{-1}. $P < 0.001$), and were more likely to have consumed narcotic/paracetamol compound analgesics (37.6% *vs.* 24.7%, $P < 0.001$). Information regarding the reasons for overdose was available for 82/110 (74.5%) of unintentional overdoses. The most common rationale for overdose was for relief of pain, including abdominal pain (n = 26), headache (n = 17), musculoskeletal pain (n = 17), toothache (n = 5), chest pain (n = 2) and dysmenorrhoea (n = 1). Other causes for overdose included accidental overdose during chemical intoxication (n = 5), non-specific systemic illness (n = 4), limb abscess (n = 2), iatrogenic overdose (n = 2) and one overdose taken unintentionally by a patient with cognitive impairment. Of the 52 subjects who had consumed compound analgesics or taken mixed overdoses unintentionally, the majority (29/52, 55.8%) had used codeine phosphate/paracetamol compounds (co-codamol), an analgesic available over the counter (OTC) in pharmacies in the UK at a dose of 8/500 mg, and only by prescription at higher doses. A total of eight subjects had overdosed on prescribed compound analgesics, namely dextropropoxyphene/paracetamol (coproxamol, n = 5) and dihydrocodeine tartrate/paracetamol (codydramol, n = 3). A total of five cases had overdosed on both co-codamol and coproxamol. The remaining compound overdose cases had used aspirin/paracetamol OTC compounds (n = 6). Of the mixed overdoses, three cases had taken non-steroidal anti-inflammatories and paracetamol, two had used aspirin and paracetamol, whilst a further three cases had taken paracetamol with benzodiazepines. The remaining six cases had taken mixed overdoses of other prescription medications and paracetamol.

Unintentional overdose patients were significantly more likely to have consumed paracetamol in a staggered fashion (90.8% *vs.* 10.6%, $P < 0.001$), and to have taken a lower cumulative paracetamol dose (11 (5–29) g *vs.* 27.5 (20–45) g, $P < 0.001$). Unintentional overdose subjects were also more likely to have a history of chronic alcohol abuse (40.8% *vs.* 20.8%, $P < 0.001$), and to have consumed alcohol with their overdose (52.5% *vs.* 42.7%, $P = 0.037$), but were less likely to have a prior psychiatric history (24.5% *vs.* 46.2%, $P < 0.001$). Unintentional overdose subjects had significantly lower admission ALT concentrations (3931 (2036–7184) IU l^{-1} *vs.* 8295 (5257–10 920) IU l^{-1}, $P < 0.001$) but had significantly more deranged serum sodium (133 (130–137) mmol l^{-1} *vs.* 136 (133–138) mmol l^{-1}, $P = 0.001$), creatinine (203 (110–327) μmol l^{-1} *vs.* 114 (81–207) μmol l^{-1}, $P < 0.001$) and albumin (31 (24–35) g l^{-1} *vs.* 37 (33–41) g l^{-1}, $P < 0.001$) concentrations. Subjects consuming paracetamol unintentionally were significantly less likely to have received treatment with NAC prior to

Table 2

Admission clinical and laboratory data in patients with intentional or unintentional paracetamol overdose

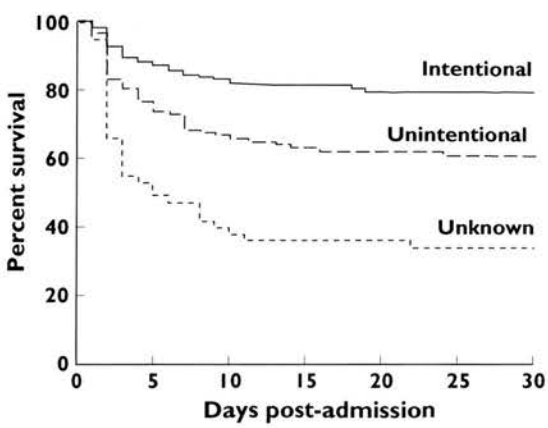
Variable		Intentional	*n*	Unintentional	*n*	*P*
Sex (male/female)		248/252 (49.6/50.4%)	500	50/60 (45.5/54.5%)	110	0.431
Age (years)		33 (24–43)		40 (30–48)		<0.001
Paracetamol concentration (mg l^{-1})		75.6 (25.4–148.2)	432	34.7 (15.9–57.5)	88	<0.001
Paracetamol dose ingested (g) (range)		27.5 (20–45) Range (4–150)	500	11 (5–29) Range (4–70)	97	<0.001
Paracetamol only		237 (48.1%)	493	43 (45.3%)	95	<0.001
Compound narcotic/paracetamol use		122 (24.7%)		38 (40%)		
Mixed overdose		134 (27.2%)		14 (14.7%)		
Associated alcohol‡		196 (42.7%)	459	52 (52.5%)	99	0.037
Alcohol abuse†		101 (20.8%)	485	42 (40.8%)	103	<0.001
Staggered overdose		53 (10.6%)	499	99 (90.8%)	109	<0.001
Previous psychiatric history		206 (46.2%)	446	23 (24.5%)	94	<0.001
Active drug use		80 (16.7%)	478	(10.5%)	105	0.110
Received NAC in referring hospital		441 (89.6%)	492	86 (80.4%)	107	0.008
Admission laboratory parameters	Platelets (x10^9 l^{-1})	129 (81–176)	500	113 (62–169)	110	0.043
	Sodium (mmol l^{-1})	136 (133–138)		133 (130–137)		0.001
	Creatinine (μmol l^{-1})	114 (81–207)		203 (110–327)		<0.001
	ALT (IU l^{-1})	8295 (5 257–10 920)		3931 (2036–7184)		<0.001
	Bilirubin (μmol l^{-1})	84 (58–112)		75 (58–118)		0.530
	Albumin (g l^{-1})	37 (33–41)		31 (24–35)		<0.001
	PT (s)	48 (34–67)		47 (33–64)		0.540
Developed encephalopathy		237 (47.4%)		65 (59.1%)		0.027
Met King's College Criteria		130 (26%)		40 (36.4%)		0.034
CVVH		141 (28.4%)		46 (41.8%)		0.048
Mechanical ventilation		190 (38%)		58 (52.7%)		0.005
Transplanted		42 (8.4%)		6 (5.5%)		0.044
Spontaneously survived		372 (74.4%)		63 (57.3%)		<0.001

Data are on admission to the SLTU unless otherwise stated and are presented as median (IQR) or numbers (%) as appropriate. †>56 units $week^{-1}$ (male); >42 units $week^{-1}$ (female). ‡Alcohol taken with paracetamol overdose.

transfer to the SLTU (80.4% *vs.* 89.6%, $P = 0.008$). Unintentional overdose patients were more likely to develop HE (59.1% *vs.* 47.4%, $P = 0.027$) and had more systemic organ failure, such as requirement for renal replacement therapy (41.8% *vs.* 28.4%, $P = 0.048$), or need for mechanical ventilation (52.7% *vs.* 38%, $P = 0.005$), than intentional overdose patients. Unintentional overdose subjects were also more likely to fulfil the KCC (36.4% *vs.* 26%, $P = 0.034$), but were subsequently less likely to undergo LT (15% *vs.* 32.3%, $P = 0.044$). Overall spontaneous survival (57.3% *vs.* 74.4%, $P < 0.001$) was significantly worse in the unintentional patient group (Figure 1).

Patients with unobtainable overdose history

In 53 (8.0%) patients, no clear history of suicidal intention (or otherwise) could be obtained, despite attempts to interview the patient, the patient's family and by liaison with the referring hospital. In the majority (72%) of cases, this was due to the patient having HE on arrival and being unable to provide a history. Of the 53 patients in whom a history was unobtainable, 19 (35.8%) were mechanically ventilated on arrival, seven (13.2%) were in grade III-IV HE and 12 (22.6%) had grade I-II HE. Compared with intentional overdose patients, 'unknown' overdose subjects had



Number of patients at risk							
Intentional	500	445	416	405	401	397	396
Unintentional	110	84	73	70	69	68	67
Unknown	53	28	22	20	19	19	18

Figure 1

Survival curves of patients with paracetamol-induced acute severe liver injury according to the pattern of overdose. Survival curves were significantly different when compared using log-rank testing ($P < 0.0001$). LT was considered equivalent to death

significantly lower admission ALT concentrations (5057 (2365–8770) IU l^{-1} *vs.* 8295 (5257–10 920) IU l^{-1}, $P < 0.001$) but had significantly more deranged serum creatinine (241 (147–362) μmol l^{-1} *vs.* 114 (81–207) μmol l^{-1}, $P < 0.001$) and albumin (32 (29–37) g l^{-1} *vs.* 37 (33–41) g l^{-1}, $P < 0.001$) concentrations. These 53 patients required significant levels of organ support, with 39 (73.6%) requiring mechanical ventilation, 29 (54.7%) renal replacement therapy and 30 (56.6%) pressor support. These patients had a particularly poor clinical outcome, with 27 (50.9%) fulfilling the KCC, 31 (58.5%) dying without transplantation and only 17 (32.1%) spontaneously surviving (Figure 1).

Prognostic accuracy of the KCC in different patterns of paracetamol overdose

The prognostic accuracy of the KCC was determined for the entire paracetamol ALF cohort (n = 344), then separately for ALF patients with intentional (n = 237) and unintentional (n = 65) overdoses (Table 3). Although the KCC had high specificity for both overdose patterns, the sensitivity in predicting outcome was considerably better for intentional overdoses (89.9%, 95% confidence intervals (CI) 83.4, 94.5) compared with unintentional overdoses (77.8%, 95% CI 62.9, 88.8).

Contraindications to LT in patients meeting the KCC according to pattern of overdose

Similar proportions of patients meeting the KCC had contraindications to LT in the intentional (74/130,56.9%), unintentional (28/40, 70%) and 'unknown' (14/27, 51.9%, P = 0.247) overdose cohorts. In the intentional cohort, 29 (39.2%) patients were rejected for listing for LT because of medical contraindications to LT, 25 (33.8%) due to active and resistant alcohol dependence and 11 (14.9%) due to a previous history of non-compliance with psychiatric therapy or a consistently stated wish to die without clinical psychiatric illness. Other reasons for not listing for LT included active intravenous drug use (six patients) and multiple episodes of self-harm (three patients). In the unintentional cohort, 14 (50%) patients were excluded because they were medically unfit to survive LT. A total of 12 (42.9%) patients were rejected due to active and resistant alcohol dependence and the remaining two (7.1%) patients due to active intravenous drug use. In the unknown cohort, six (42.9%) patients were excluded due to alcohol abuse, five (35.7%) patients due to medical contraindications and three (21.4%) patients for psychosocial reasons.

Temporal changes in the patterns of overdose

To determine if there had been temporal changes in the overdose pattern over the period of observation, we compared the initial 5 complete years of the observation period (1993–97) with the last 5 complete years of the observation period (2003–07). Fewer patients were admitted with paracetamol-induced acute liver injury in the later cohort (179 patients) compared with the earlier cohort (240 patients). However, a significantly greater proportion of the patients in the later cohort had overdosed unintentionally (1993–97: 27/240 patients, 11.3%; 2003–07: 37/179 patients, 20.7%; P = 0.009).

Predictors of death in paracetamol-induced acute liver injury

In view of the significant differences in a number of prognostic variables between the different overdose subgroups, logistic regression analysis of SLTU admission parameters including patterns of overdose was performed to determine independent predictors of death or LT in paracetamol-induced acute severe liver injury (Table 4). Univariate analysis identified increasing age ($P < 0.001$), WCC ($P < 0.001$), PT ($P < 0.001$), serum creatinine ($P < 0.001$), H^+ ($P < 0.001$), and hyponatraemia (P = 0.011) as potential predictors of death/LT. Unintentional overdoses (P = 0.001), the absence of clinical history ($P < 0.001$), the presence of any grade of encephalopathy on admission ($P < 0.001$) and thrombocytopaenia ($P < 0.001$) were also potentially significant after univariate analysis. Acute or chronic alcohol abuse was not predictive of a poorer outcome, nor was lack of treatment with NAC in the referring hospital. Multivariate analysis identified the presence of encephalopathy on admission (odds ratio (OR) 4.50, 95% CI 2.76, 7.34), increasing WCC (OR 1.04, 95% CI 1.02, 1.06), admission PT (OR 1.03, 95% CI 1.02, 1.04), and admission creatinine (OR 1.00, 95% CI 1.00, 1.01) as independently predictive of death/LT. Both unintentional overdoses (OR 1.91, 95% CI 1.07, 3.43), and overdoses where there was no reliable history (OR 6.65, 95% CI 1.78, 24.81) were independently predictive of a poor outcome. Other independent predictors were

Table 3

Prognostic accuracy of the KCC in paracetamol-induced ALF

	KCC+ve/ deaths	Total deaths	Sensitivity (95% CI)	Specificity (95% CI)	LR+ (95% CI)	LR– (95% CI)	DOR (95% CI)
Paracetamol ALF cases (n = 344)	197/180	213	84.5 (81.3, 87.0)	87.0 (81.8, 91.1)	6.5 (4.5, 9.8)	0.18 (0.14, 0.23)	36.6 (19.5, 68.4)
Intentional (n = 237)	126/116	129	89.9 (83.4, 94.5)	90.7 (83.6, 95.5)	9.7 (5.4, 17.6)	0.11 (0.07, 0.19)	87.4 (36.7, 208.1)
Unintentional (n = 65)	38/35	45	77.8 (62.9, 88.8)	85.0 (62.1, 96.8)	5.2 (1.8, 14.9)	0.26 (0.15, 0.47)	19.8 (4.8, 81.6)

Transplanted patients have been included as having died. +LR/–LR: +ve/–ve likelihood ratio; DOR: diagnostic odds ratio.

Table 4

Factors predictive of mortality on univariate and multivariate analysis of admission parameters in patients with paracetamol-induced acute severe liver injury

Variable	Univariate OR (95% CI)	*P* value	Multivariate OR (95% CI)	*P* value
Unintentional overdose	1.29 (1.09, 1.53)	0.001	1.91 (1.07, 3.43)	0.032
Overdose history unavailable	2.52 (1.98, 3.21)	<0.001	6.65 (1.78, 24.81)	0.005
Age	1.04 (1.02, 1.05)	<0.001	1.04 (1.02, 1.06)	<0.001
Concomitant alcohol with OD	1.00 (1.00, 1.00)	0.527	NA	
Chronic alcohol abuse	1.34 (0.79, 2.26)	0.274	NA	
Not given NAC in referring hospital	1.37 (0.84, 2.24)	0.210	NA	
Encephalopathy on admission (any grade)	5.50 (3.55, 8.53)	<0.001	4.50 (2.76, 7.34)	<0.001
Admission WCC	1.11 (1.08, 1.15)	<0.001	1.04 (1.02, 1.06)	<0.001
Admission platelet count	0.99 (0.99, 1.00)	<0.001	0.99 (0.99, 1.00)	0.012
Admission PT	1.03 (1.02, 1.03)	<0.001	1.03 (1.02, 1.04)	<0.001
Admission sodium	0.96 (0.92, 0.99)	0.011	0.99 (0.94, 1.03)	0.572
Admission creatinine	1.01 (1.01, 1.01)	<0.001	1.00 (1.00, 1.01)	<0.001
Admission H^+	1.08 (1.06, 1.10)	<0.001	1.08 (0.97, 1.21)	0.180

LT was considered equivalent to death.

increasing age (OR 1.04, 95% CI 1.02, 1.06), and thrombocytopaenia (OR 0.99, 95% CI 0.99, 1.00).

Discussion

In this large cohort study of paracetamol-induced acute severe liver injury we have analysed the impact of suicidal ideation upon patient outcome. Using prospective definitions of overdose pattern, intentional (suicidal) overdose was the commonest pattern of paracetamol ingestion, accounting for 75.4% of all paracetamol cases. However, despite lower admission paracetamol and ALT concentrations, patients with unintentional overdose had significantly reduced survival compared with intentional overdoses. Additionally, the KCC were less sensitive in predicting outcome in unintentional overdose cases. Both unintentional overdoses and 'unknown' overdoses, where no clear history could be obtained, were independently associated with increased mortality. These data suggest that the pattern of overdose should be taken into account when assessing patients with paracetamol-induced hepatotoxicity, and that, irrespective of their admission paracetamol concentrations, those patients with unintentional overdose should be managed as high-risk cases due to their significantly increased mortality.

The strengths of this study include the large number of patients, the single centre nature of the study and the prospectively defined criteria of overdose. The SLTU represents a single referral and management facility for all patients in Scotland with ALF irrespective of their suitability for LT, and the Scottish population has remained relatively stable at 5.1 million over the period of the study. However, we recognize that not all ALF cases occurring in Scotland will have been transferred to the SLTU during the course of the study due to medical instability precluding safe patient transfer [13]. Criteria for patient admission have remained largely unchanged during the time course of the study, further reducing patient heterogeneity, a recognized problem in previous cohort studies of paracetamol hepatotoxicity [14, 15]. Our overall mortality rate of 32.7% represents selection bias for the more severe paracetamol cases in Scotland, since admissions to the SLTU are determined by severity of liver dysfunction, rather than on the basis of a history of paracetamol consumption or number of tablets consumed. This latter point is of particular note since intentional and unintentional paracetamol overdoses represent considerably different patient populations with regards to demographics, timing of presentation and degree of organ dysfunction at presentation. Suicidal patients often present to a hospital setting as a direct result of the psychological consequences of their overdose, rather than as a result of symptoms; in contrast, unintentional overdoses usually present because of morbidity and therefore tend to be systemically unwell at presentation. Our study partially eliminates this problem through a gatekeeper mechanism, but inevitably introduces referral bias, since only those patients with potentially reversible organ failure are accepted.

Defining paracetamol as a cause of hepatotoxicity is recognized as particularly difficult in cases where there is no obvious suicidal intent or patients are unable to give a history. We prospectively defined paracetamol hepatotoxicity and overdose subgroups, but acknowledge that some of the unintentional overdoses in our cohort may represent other, unidentified, primary causes of hepatotoxicity in whom paracetamol was taken to relieve systemic malaise. However, paracetamol overdose was not simply used as a 'catch-all' diagnosis in the absence of an alternative diagnosis. Seronegative hepatitis represented 63/938 (6.7%) of all cases during this study, none of whom were classified as paracetamol-induced hepatotoxicity (data not

shown). We also recognize that some unintentional cases will have been disguised suicides, especially since 'accidental' overdoses are associated with both chronic alcohol abuse and underlying depression [7]. Indeed, our analysis would suggest potential suicidal motives behind large overdoses of paracetamol, particularly if not taken as a compound analgesic. However, we were careful to ascertain as much information as possible regarding prior suicidal ideation, timings, and circumstances of overdose, associated acute and chronic alcohol consumption, and previous psychiatric history before classifying each overdose to a particular subgroup. This assessment included experienced psychiatric review. The ability and time available to obtain a detailed psychiatric history to confirm intent is limited by the rapid clinical progression of paracetamol-induced ALF, and by the development of HE, but, given that the lack of a detailed history regarding suicidal intent is in itself an independent predictor of poor prognosis, this further emphasizes that such cases should be treated as high risk.

Previous studies have suggested that accidental paracetamol overdose is associated with increased mortality [6, 14]. However, this poorer prognosis is not a universal finding in other cohort studies of paracetamol hepatotoxicity [2, 7]. The recently published multicentre cohort analysis from the US Acute Liver Failure Study group found similar survival in 131 unintentional patients (72% 3 week survival) compared with 122 intentional patients (71% 3 week survival) [2]. This difference in survival compared with our data does not relate to differences in transplant rates, but may be due to the increased frequency of renal dysfunction in our cohort compared with the US series. We have recently reported that renal dysfunction on admission in patients with ALF is a significant predictor of poor outcome [16]. Another important potential confounding factor is alcohol abuse, given that unintentional overdose patients in our cohort were not only more likely to abuse alcohol chronically, but were also more likely to consume alcohol acutely at the time of overdose. However, neither acute nor chronic alcohol abuse was an independent predictor of death or LT on multivariate analysis. The association of alcoholism with other confounding factors, such as delayed presentation, increased paracetamol dosage, and older age, may account for the poorer outcomes previously reported in alcoholics following paracetamol overdose [17, 18]. Furthermore, since alcoholism is a recognized risk factor for disguised suicidal overdose [19], some parasuicidal attempts in alcoholic patients may have been misclassified as unintentional overdoses. Another important element in the prognosis of unintentional overdoses is likely to be the time period prior to treatment with NAC ('time to NAC'). Our data demonstrate that unintentional overdoses are less likely to receive NAC in the referring hospital, although this was not an independent risk factor for poor outcome. Delayed 'time to NAC' is a recognized independent risk factor for poor outcome following single time point paracetamol overdose [18], and delayed presentation is thought to be more common amongst alcoholics [20]. Given the increased frequency of both alcoholism and staggered overdoses amongst the unintentional overdose cohort, it is likely that absence of, or delayed treatment with, NAC in the referring hospital was at least partially responsible for the poorer outcome in this group. As a result, we would suggest that patients with acute liver injury and a history of supratherapeutic paracetamol ingestion should be treated as high risk irrespective of serum paracetamol concentrations, and commenced on NAC treatment whilst other aetiological investigations are pending.

Admission HE, coagulopathy, renal failure, thrombocytopaenia, leucocytosis and increasing age, as well as unintentional or unknown patterns of overdose, predicted a poorer outcome in the paracetamol overdose patients as a whole. Many of these factors are well established as predicting a poorer outcome in paracetamol-induced ALF [11], and the increasingly recognized deleterious effects of the systemic inflammatory response following paracetamol hepatotoxicity may explain the predictive nature of leucocytosis seen in this study [21–23]. Increasing age has previously been recognized as an independent risk factor for poor outcome following paracetamol overdose [24], and the older age of the unintentional overdose cohort may represent either an increased frequency of this deleterious overdose pattern amongst older patients, or a lower threshold for the development of severe hepatotoxicity following paracetamol overdose in older subjects.

We have observed an increased frequency of unintentional overdose in our cohort compared with data previously published from King's College in which only 8% of paracetamol overdoses were due to accidental ingestion [5]. Due to the potential selection bias associated with our study, we cannot conclude that the nationwide incidence of unintentional paracetamol overdoses is increasing, but this question deserves attention due to the poor outcome seen in this overdose subgroup. The increased proportions of unintentional overdoses seen in this study compared with the King's College study may also reflect temporal changes associated with legislation affecting the availability and packaging of paracetamol that followed the publication of the King's College series [25], particularly given the increased proportion of this overdose type seen in the later years of this cohort study. Alternative explanations for these differences include socioeconomic factors and the increased rates of chronic alcohol abuse in the Scottish population compared with the rest of the UK [26]. The KCC identify patients with increased risk of death following paracetamol poisoning and are the 'transplant criteria' in use throughout the UK to determine patient prognosis [11, 12]. Within our unintentional cohort the KCC are specific, but lack sensitivity, for paracetamol-induced ALF, suggesting that alternative prognostic criteria may be required following this pattern of overdose [27–33].

In conclusion, unintentional paracetamol overdose is independently associated with increased mortality compared with intentional overdose. This pattern of overdose is associated with older age, acute and chronic alcohol abuse and a staggered pattern of overdose. Despite lower admission ALT and paracetamol concentrations, unintentional overdose patients have increased systemic dysfunction and poorer clinical outcomes compared with intentional overdoses. The KCC are less sensitive in predicting outcome in unintentional overdose and alternative prognostic criteria may be required.

Competing Interests

There are no competing interests to declare.

We are grateful for the support of the other members of the medical and nursing team in the management of these patients. There was no financial support for this study.

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AP&T Alimentary Pharmacology and Therapeutics

The systemic inflammatory response syndrome and sequential organ failure assessment scores are effective triage markers following paracetamol (acetaminophen) overdose

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Publication data
Submitted 21 February 2011
First decision 20 March 2011
Resubmitted 31 March 2011
Accepted 18 April 2011

SUMMARY

Background

The systemic inflammatory response syndrome (SIRS) and sequential organ failure assessment (SOFA) scores are widely used as prognostic markers in critical care settings and could improve triage of high-risk paracetamol (acetaminophen) overdose patients.

Aim

To evaluate the prognostic accuracy of the SIRS and SOFA scores following single time point paracetamol overdose.

Methods

Analysis of 100 single time point paracetamol overdoses admitted to a tertiary liver centre, with subsequent prospective validation of identified thresholds. Individual laboratory samples were correlated with the corresponding clinical parameters in relation to time post-overdose, and the daily SOFA and SIRS scores calculated.

Results

A total of 74 (74%) patients developed the SIRS, which occurred significantly earlier in patients who died ($n = 21$) compared with spontaneous survivors ($n = 53$, $P = 0.05$). The SIRS occurred in 70 (70%) patients by 96 h post-overdose, with a 30% mortality rate; compared with 0% mortality in the 30 non-SIRS patients ($P = 0.001$). Median SOFA scores were significantly higher in nonsurvivors at 48 ($P = 0.009$), 72 ($P < 0.001$), and 96 h ($P < 0.001$). A SOFA score >7 during the first 96 h post-overdose predicted death/transplantation with a sensitivity of 95.0 (95% CI 78.5–99.1) and specificity of 70.5 (95% CI 66.3–71.6). A validation cohort of 38 single time point paracetamol overdoses confirmed the extremely high negative predictive value of both the SIRS and SOFA thresholds.

Conclusions

The absence of either a SOFA score >7 or a SIRS response during the first 96 h following paracetamol overdose could improve triage and reduce transfers of lower risk patients to tertiary liver centres.

doi:10.1111/j.1365-2036.2011.04687.x

INTRODUCTION

Paracetamol (acetaminophen) overdose remains the main aetiological factor responsible for acute liver failure (ALF) in the developed world.[1] The only effective treatment for the condition remains emergency orthotopic liver transplantation (OLT).[2] Paracetamol-induced ALF is strongly associated with the development of the systemic inflammatory response syndrome (SIRS) and a subsequent cascade of complications including hepatic encephalopathy (HE), coagulopathy and multiple organ dysfunction syndrome.[3] In human studies, development of the SIRS following paracetamol-induced hepatotoxicity has been associated with both an increased risk of worsening HE grade,[4] and with poorer overall outcome, irrespective of the presence of infection.[3] Existing prognostic systems, such as the King's College Criteria (KCC), utilise features of HE and extrahepatic organ injury to predict a hopeless prognosis without OLT, but their use has been criticised for their relatively low negative predictive value[5] and by the inherent requirement for multiorgan failure to have developed before considering definitive treatment. Several studies have therefore examined the prognostic accuracy of multiorgan failure assessment scores such as the Sequential Organ Failure Assessment (SOFA) and Acute Physiology and Chronic Health Evaluation (APACHE) II scores to predict outcome following paracetamol hepatotoxicity,[6–8] but these studies have tended to apply the prognostic test in question at the point of hospital admission, rather than in relation to the time from overdose.[9] This inevitably introduces temporal confounding, as many patients present to hospital following paracetamol overdose due to psychological, rather than physical, morbidity. Therefore, a better understanding of the temporal evolution of the SIRS and multiorgan failure following paracetamol overdose could improve prognostication in this condition and could aid early identification of high-risk patients.

To our knowledge, no studies exist which examine the temporal relationship between the initial hepatotoxic insult (i.e. paracetamol overdose) and development of the SIRS and multiorgan failure. The aims of this study were to examine these temporal changes in a cohort of patients who required tertiary level care after taking a single intentional paracetamol overdose. It was hypothesised that the absence of a SIRS response or extrahepatic organ injury following overdose would be associated with a low risk of in hospital mortality and that the SIRS and SOFA scores could therefore act as quantitative triage instruments to identify those patients at lower risk of requiring emergency OLT.

PATIENTS, METHODS AND DEFINITIONS

Since its inception in 1992, the Scottish Liver Transplantation Unit (SLTU) has prospectively collected and maintained a dedicated database of all acute liver injury patients admitted to the unit. Data recorded in this database include details of patient history, clinical examination and laboratory results along with therapeutic interventions, including intensive care admission, need for renal replacement therapy (RRT) or inotropic support. Where possible the paracetamol preparation, number of tablets, type (whether intentional, accidental, single time point or staggered) and timing of overdose, delay to presentation and use of *N*-acetyl cysteine (NAC) are all recorded. The cohort analysed were 100 consecutive single time point intentional [defined as a paracetamol overdose (>4 g) taken at a single defined time point with the objective of self-harm] paracetamol overdoses admitted to the SLTU with acute liver injury between April 2003 and February 2009 and a prospectively collected cohort of 38 patients admitted between March 2009 and July 2010. Ethical approval for the study was obtained from the Scotland 'A' Research and Ethics Committee. We specifically excluded patients with staggered overdoses or overdoses taken accidentally in an attempt to relieve pain, because of the confounding temporal and diagnostic problems associated with these overdoses.[10]

Paracetamol overdose was defined as at least 2/3 of: a history of ingestion of potentially toxic amounts of paracetamol (>4 g/day); detection of paracetamol in the serum >10 mg/L; or a serum alanine aminotransferase (ALT) level >1000 IU/L within 7 days of a history of paracetamol ingestion irrespective of the serum paracetamol concentration.[1] All 138 patients fulfilled all of these three criteria. Severe acute liver injury was defined as sudden deterioration in liver function with associated coagulopathy in the absence of a history of chronic liver disease, while the term ALF (i.e. fulminant liver failure) was restricted to those patients developing HE.[11] Guidelines for accepting patients from referring hospitals were based on previously published criteria from the British Society of Gastroenterology and remained unchanged over the time course of the study.[12] Patients admitted to the SLTU are managed using a standard protocol as previously described,[13] the main goals of which have remained similar over the duration of this cohort study. The KCC are used in this unit and throughout the UK to determine patients who will most likely die without OLT.[14] As the purpose of this study was not to develop

transplantation listing criteria, death and OLT were considered equivalent when undertaking survival analysis.

Laboratory parameters

Retrospective analysis of all hospital electronic laboratory records was performed to obtain all standard haematological, biochemical and coagulation samples obtained for each patient during the first 10 days of admission. Each sampling time point was noted from either the clinical notes and/or the laboratory receipt and the corresponding haemodynamic and clinical parameters for each sampling time point retrieved from the clinical notes. These data were then individually compared against the reported time from overdose for each patient, and the corresponding laboratory and clinical values recorded in a dedicated database.

Systemic inflammatory response syndrome

The presence of the SIRS was defined as two or more of: temperature <36 °C or >38 °C, heart rate >90 beats/min, leucocyte count $<4 \times 10^9$/L or $>12 \times 10^9$/L and tachypnoea >20 breaths/min or $PaCO_2 < 4.3$ kPa.[15] The daily presence or absence of the SIRS and the number of the SIRS components fulfilled were retrospectively calculated for each patient by correlating the daily leucocyte count with the most abnormal temperature and heart rate recorded during each corresponding day of admission. Respiratory rate was only included for nonventilated patients, as the majority of patients in our unit are electively mechanically ventilated following the development of grade III or IV HE.

Sofa score and organ failure

Organ failure was assessed using the SOFA score, which assesses six organ systems – hepatic; renal; coagulation; cardiovascular; respiratory; and central nervous – and provides a graded score from 0 to 4 points for each organ system (Table 1).[16] The SOFA score was calculated for each 24-h period following overdose, using the most deranged values for each parameter in each 24 h period. Organ failure was defined as a SOFA score ≥3 points for the organ concerned (Table 1). As a result of potential confounding, SOFA scores were not calculated following the administration of platelet transfusions.

Statistical analysis

Statistical analysis was performed using SPSS (SPSS 16.0, Chicago, IL, USA) and GRAPHPAD PRISM (GraphPad Software Inc., La Jolla, CA, USA). Data values are presented as median ± interquartile range (IQR) or percentages unless otherwise stated. When undertaking survival analysis, death and OLT were considered equivalent. Receiver operating characteristic (ROC) analysis was used to identify optimum threshold values to discriminate nonsurvi-

Table 1 | The sequential organ failure assessment (SOFA) score

SOFA score

	Score				
Organ system	0	1	2	3	4
Respiratory: PaO_2/FiO_2	>400	≤400	≤300	≤200	≤100
Renal: creatinine, μmol/L (mg/dL)	≤110 (≤1.2)	110–170 (1.2–1.9)	171–299 (2.0–3.4)	300–440 (3.5–5.0); urine output ≤500 mL/day	>440 (>5.0); urine output <200 mL/day
Hepatic: bilirubin, μmol/L (mg/dL)	≤20 (≤1.2)	20–32 (1.2–1.9)	33–101 (2.0–5.9)	102–204 (6.0–12.0)	>204 (>12.0)
Cardiovascular: hypotension	No hypotension	MAP <70 mmHg	Dopamine ≤5*, dobutamine (any dose)	Dopamine >5* or epinephrine ≤0.1* or nor epinephrine ≤0.1*	Dopamine >15* or epinephrine >0.1* or norepinephrine >0.1*
Haematological: platelet count	>150	≤150	≤100	≤50	≤20
Neurological: Glasgow Coma Scale score	15	13–14	10–12	6–9	<6

FiO_2, fractional inspired oxygen; MAP, mean arterial pressure; PaO_2, arterial oxygen tension.

* Adrenergic agents administered for at least 1 h (doses given are in μg/kg per minute).

vors. Continuous data were compared using either analysis of variance or the Kruskal–Wallis test if inter-group variances were unequal, with *post hoc* Dunn's testing used to compare selected groups. Categorical data were analysed using Chi-squared tests or Fishers exact test. Bonferroni corrections were undertaken for repeated measures. Missing data were treated in a last observation carried forward manner. Results were considered statistically significant when $P < 0.05$.

RESULTS

Patients and details of overdose

A total of 100 patients (46 men, 54 women) admitted to the SLTU between April 2003 and February 2009 were included in the study. During this period, a total of 347 patients had been admitted, of whom 111 were classified as 'nonparacetamol' cases, 62 patients had taken a staggered paracetamol overdose, 45 patients had overdosed on paracetamol accidently and in the remaining 29 cases, the details of the paracetamol overdose were unclear. The median patient age of the 100 included patients was 34 (IQR 24-43) years. A total of 83 patients were transferred to the SLTU from outlying health boards, at a median time of 50 (36–66.5) h following overdose. The remaining patients were admitted to the SLTU from wards within the Royal Infirmary of Edinburgh or from local hospitals. The median ingested paracetamol dose was 25 (17.5–41) g. All 100 patients received NAC treatment at their local hospital, at a median time from overdose of 22.75 (9.5–42.25) h. A total of 47 (47%) of patients had taken a mixed overdose. As shown in Table 2, none of these demographic parameters or potential confounders differed significantly between survivors and patients who died/required OLT. Other baseline demographic and clinical characteristics of the paracetamol study group are shown in Table 2.

Hepatic encephalopathy and other outcomes

A total of 29 (29.0%) of paracetamol-induced acute liver injury patients were encephalopathic on admission to the SLTU and a further 18/71 (25.4%) went on to develop HE during admission. A total of 47 (47%) patients therefore developed HE and thus ALF, at some point during their illness. Of these patients, 22 (46.8%) subsequently met the modified KCC, at a median time from overdose of 51.5 (39.0–73.0) h from overdose. Of these 22 patients, 3 were transplanted, 14 died without OLT and 5 recovered spontaneously. Four patients died without meeting the modified KCC. The sensitivity, specificity and diagnostic odds ratios (DOR) of the KCC were 81.0 [95% confidence intervals (CI) 65.0–90.7], 93.7 (95% CI 89.4–96.3) and 62.9 (95% CI 15.7–251.3) respectively. Of the nontransplanted patients who met the KCC, 13 patients were excluded from listing for OLT because of active and resistant alcohol dependence (11 patients) and active intravenous drug abuse (2 patients). Four patients were listed but subsequently deemed medically unfit to survive OLT, while a further two patients showed clear signs of spontaneous recovery and were delisted prior to an organ becoming available. On admission, patients who later died or were transplanted were significantly more coagulopathic [PT 80 (53–103) s vs. 48 (33–70) s, $P = 0.002$], had lower serum albumins (30 (23–35) g/L

Table 2 | Demographics of study population and details of paracetamol overdose

Variable	Dead/OLT	*n*	Spontaneous survival	*n*	*P*
Gender (male/female)	6/15 (28.6%/71.4%)	21	40/39 (50.6%/49.4%)	79	0.071
Age (years)	37 (28-44)		33 (22-43)		0.115
Ingested paracetamol dose (g)	27.5 (15.5-40.0)		25.0 (17.0-40.5)		0.226
Admission paracetamol level (μmol/L)	56 (37-146)		70 (24-145)		0.545
Mixed OD	8 (38.1%)		39 (49.4%)		0.115
Previous OD	8 (57.1%)	14	29 (46.0%)	63	0.388
Active drug abuse	3 (14.3%)	21	11 (14.5%)	76	0.654
Weekly alcohol consumption (units)	50 (4-140)	13	20 (2-68)	57	0.182
Time from OD to NAC (h)	15.5 (10.0-34.5)	21	23.5 (11.0-43.5)	79	0.791
Time from OD to SLTU admission (h)	41.0 (29.5-49.0)		53.0 (45.5-67.0)		0.045

Data are presented as median (± interquartile range) or percentages as appropriate.

vs. 34 (31–37) g/L, $P = 0.013$) and had significantly higher serum creatinine levels [174 (118–239) μmol/L vs. 94 (75–145) μmol/L, $P = 0.006$] compared with spontaneous survivors. A significantly greater proportion of patients who died or were transplanted required multiple organ support, such as RRT, inotropic support and mechanical ventilation (Table S1).

SIRS and temporal relationship to overdose

Examination of the cumulative incidence of the SIRS revealed that by 48 h post-overdose, 27/40 (67.5%) patients had mounted a SIRS response, with a mortality rate in the SIRS group of 44.4%, compared with 7.7% for those patients not mounting a SIRS response (Fisher's $P = 0.020$). By 72 h post-overdose, 59/86 (68.6%) patients had developed a SIRS during admission, with a mortality rate of 28.8%, compared with a mortality rate of only 3.7% (1/27 patients, Fisher's $P = 0.007$) in patients without a SIRS at this point. By 96 h, the cumulative incidence of the SIRS had risen to 70 (70%) patients, with a mortality rate of 30% in this group; compared with a mortality rate of 0% in the 30 patients without the SIRS (Fisher's $P = 0.001$). The sensitivity, specificity, negative predictive values and DORs of the SIRS occurring by each time point post-overdose are shown in Table 3. In total, 74 (74%) of patients developed the SIRS post-overdose, but this occurred significantly earlier following overdose in patients who died [median 43.0 (28.5–61.0) h, $n = 21$] compared with patients who survived [60.0 (36.5–81.0) h, $n = 53$, $P = 0.05$]. By contrast, the presence of the SIRS at the time of hospital admission, or at the development of HE, resulted in reduced sensitivity and DORs compared with the values obtained at 48, 72 and 96 h post-overdose (Table 3). The number of SIRS components fulfilled following overdose was significantly related to increasing mortality at 48 ($P = 0.047$), 72 ($P = 0.0057$) and 96 ($P < 0.0001$) h post-overdose.

Temporal relationship between overdose and SOFA score

SOFA scores could be calculated for 99/100 patients. SOFA scores ranged from 1 to 15 by 48 h post-overdose, 0 to 17 by 72 h and 1 to 18 by 96 h post-overdose. Median SOFA score (± IQR) was significantly higher in patients who died or were transplanted at each of these three time points [48 h: 9 (6.25–12.25) vs. 5 (3–7), $P = 0.009$; 72 h: 12.5 (8.75–17) vs. 5 (3–7), $P < 0.001$; 96 h: 15 (9–19) vs. 5 (3–8), $P < 0.001$]. The number of organ systems failing by both 72 ($P = 0.0003$) and 96 ($P < 0.0001$) h post-overdose was significantly related to mortality, which ranged from 5.9% for patients without any organ system failure at 72 h post-overdose to 71.4% for those patients with three or more organ system failures at this time point, Figure 1. ROC analysis of SOFA scores showed an area under the curve of 0.80 (95% CI 0.65–0.95), 0.90 (95% CI 0.82–0.97) and 0.91 (95% CI 0.85–0.98) at 48, 72 and 96 h post-overdose respectively. The optimal SOFA score thresholds for discriminating nonsurvivors at these three time points were >4 at 48 h, >6 at 72 h and >7 at 96 h respectively, Table 4.

Table 3 | Predictive accuracy of the presence of the systemic inflammatory response syndrome (SIRS) following single time point paracetamol overdose

Time from overdose (h)	*N*/deaths	Cumulative incidence of SIRS/deaths	Sensitivity (95% CI)	Specificity (95% CI)	Negative predictive value (95% CI)	Diagnostic odds ratio (95% CI)
48	40/13	27/12	92.3 (64.0–99.8)	44.4 (25.5–64.7)	92.3 (71.9–98.6)	9.6 (1.1–84.6)
72	86/18	59/17	94.4 (76.7–99.0)	38.2 (33.5–39.4)	96.3 (84.5–99.3)	10.5 (1.7–64.8)
96	100/21	70/21	100.0 (83.9–100.0)	38.0 (27.3–49.6)	100.0 (90.3–100.0)	∞ (3.3–∞)
At SLTU admission	100/21	52/17	81.0 (62.4–92.1)	55.7 (50.8–58.7)	91.7 (83.6–96.5)	5.3 (1.7–16.5)
At onset of HE	47/21	30/18	85.7 (70.7–94.6)	53.8 (41.7–61.0)	82.4 (63.8–93.4)	7.0 (1.7–27.6)
Modified KCC	100/21	KCC +ve/ deaths 22/17	81.0 (65.0–90.7)	93.7 (89.4–96.3)	94.9 (90.6–97.5)	62.9 (15.7–251.3)

CI, confidence intervals; HE, hepatic encephalopathy; SLTU, Scottish Liver Transplantation Unit.

The SIRS was defined as the presence of ≥2 SIRS components in any 24-h period. The accuracy of the SIRS is compared with the modified King's College Hospital poor prognostic criteria (KCC).

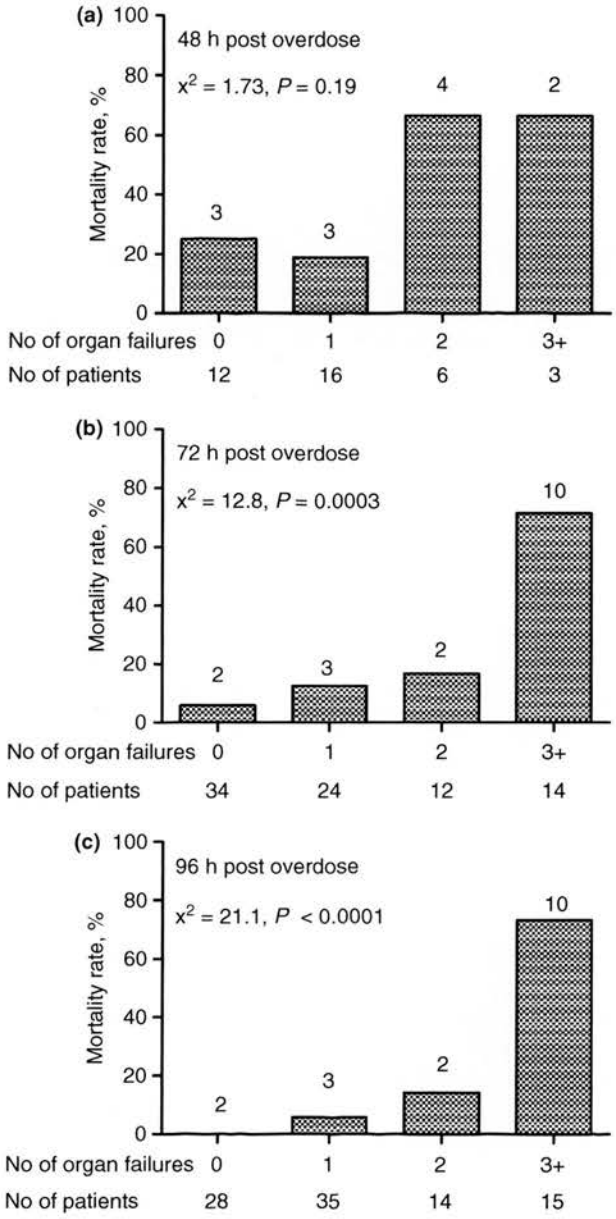

Figure 1 | Association between mortality rate and the number of organ failures identified by the sequential organ failure assessment (SOFA) score at (a) 48, (b) 72 and (c) 96 h following single time point paracetamol overdose. Numbers above the bars indicate number of deaths. Death and liver transplantation were considered equivalent.

A SOFA score >7 at any time during the first 96 h post-overdose predicted outcome with excellent accuracy, with a significantly higher median maximum SOFA score among patients who died [17 (12–18.75)] compared with spontaneous survivors [6 (3–8), $P < 0.0001$] and an area under the ROC of 0.92 (95% CI 0.86–0.98). Further analysis using this SOFA threshold of >7 during the first 96 h post-overdose revealed that only 2/56 (3.6%) patients not fulfilling this criteria required RRT and only 1/56 (1.8%) patients required intracranial pressure monitoring.

A previous report also evaluated SOFA scores following paracetamol overdose and reported that a SOFA score >8 early after admission and >12 at the onset of HE were the most discriminatory thresholds for determining prognosis.[8] At admission, the area under the ROC for SOFA was 0.80 (95% CI 0.68–0.93), but using the previously reported threshold of >8 to predict a poor prognosis resulted in a low sensitivity of 38.1 (95% CI 23.7–48.7). The area under the ROC for a SOFA >12 at the onset of HE ($n = 47$) was 0.71 (95% CI 0.56–0.86), with a low sensitivity of 23.8 (95% CI 12.2–34.2).

SOFA score and outcome

Univariate analysis of the individual components of the SOFA score at 72 and 96 h post-overdose was performed to identify those components which best predicted an adverse outcome (Table S2). All the organ failure components except the hepatic failure element were strongly associated with mortality, so a modified SOFA score, which excluded points for hepatic failure, was computed. ROC analysis of this modified SOFA score showed an area under the curve of 0.81 (95% CI 0.67–0.95), 0.90 (95% CI 0.84–0.97) and 0.91 (95% CI 0.84–0.98) at 48, 72 and 96 h post-overdose respectively. A modified SOFA threshold score of >3 at 48 h and >4 at 72 h, improved upon the specificity of the original SOFA score at both these time points without affecting sensitivity. A modified SOFA threshold score of >5 at any point during the first 96 h following overdose was highly predictive of death or OLT, with a sensitivity of 95.0 (95% CI 78.7–99.1), specificity of 76.9 (95% CI 72.8–78.0) and negative predictive value of 98.4 (95% CI 93.0–99.7) (Table 4).

Association between SIRS and SOFA scores

We then explored whether those patients with high SOFA scores also manifested a SIRS response. By 96 h post-overdose, the median (± IQR) SOFA score in those patients who manifested a SIRS response was significantly higher [8 (5–15)] compared with those patients with no SIRS at that point [5 (3–6.25), $P = 0.0011$]. There was a strong correlation between these two variables (Spearman's $r = 0.474$, $P < 0.0001$), as shown in Figure 2, with those patients who died tending to have both a SIRS response and a higher SOFA score than spontaneous survivors.

Table 4 | Predictive accuracy of the Sequential Organ Failure Assessment (SOFA) score following single time point paracetamol overdose

Time from overdose (h)	Threshold SOFA score	*N*/ deaths	SOFA > threshold/ deaths	Sensitivity (95% CI)	Specificity (95% CI)	Negative predictive value (95% CI)	Diagnostic odds ratio (95% CI)
48	>4	37/12	25/11	91.7 (61.5-99.8)	44.0 (24.4-65.1)	91.7 (70.2-98.5)	8.6 (1.0-77.5)
72	>6	84/18	40/17	94.4 (72.7-99.9)	65.2 (52.4-76.5)	97.7 (90.4-99.6)	31.8 (4.0-254.2)
96	>7	92/15	35/14	93.3 (68.1-99.8)	72.7 (61.4-82.3)	98.2 (92.8-99.7)	37.3 (4.6-301.8)
Up to 96	>7	98/20	42/19	95.0 (78.5-99.1)	70.5 (66.3-71.6)	98.2 (92.3-99.7)	45.4 (7.2-278.7)
48 (Modified SOFA)	>3	37/12	19/11	91.7 (61.5-99.8)	56.0 (34.9-75.6)	94.4 (79.9-99.0)	23.4 (3.1-160.4)
72 (Modified SOFA)	>4	84/18	33/17	94.4 (72.7-99.9)	75.8 (63.6-85.5)	98.0 (91.9-99.6)	53.1 (8.2-332.1)
96 (Modified SOFA)	>4	92/15	38/14	93.3 (68.1-99.8)	70.1 (58.6-80.0)	98.1 (92.3-99.7)	31.7 (4.9-196.7)
Up to 96 (Modified SOFA)	>5	98/20	37/19	95.0 (78.7-99.1)	76.9 (72.8-78.0)	98.4 (93.0-99.7)	63.3 (9.9-391.4)
Modified KCC	N/A	100/21	KCC +ve/ deaths 22/17	81.0 (65.0-90.7)	93.7 (89.4-96.3)	94.9 (90.6-97.5)	62.9 (15.7-251.3)

CI, confidence intervals; HE, hepatic encephalopathy; SLTU, Scottish Liver Transplantation Unit; N/A, not applicable.

The accuracy of the SOFA score is compared with the modified King's College Hospital poor prognostic criteria (KCC). The modified SOFA score excludes the hepatic failure component.

Optimal thresholds for predicting death or liver transplantation were derived from receiver operator characteristic analysis.

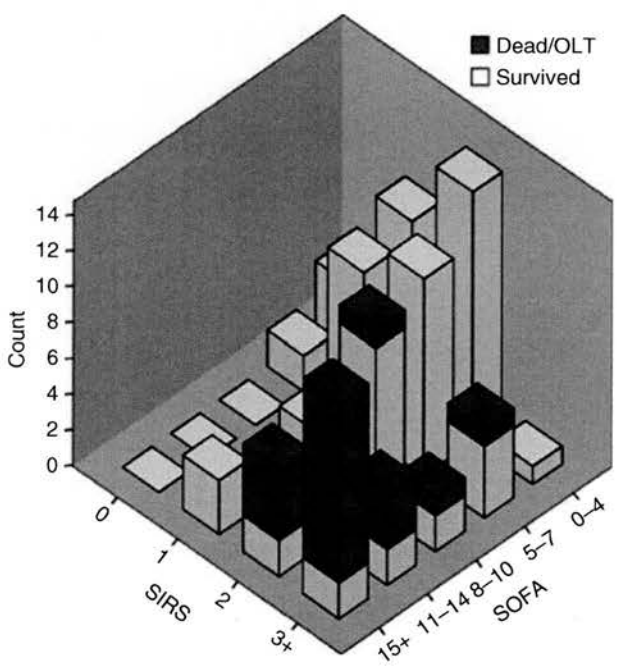

Figure 2 | Association between sequential organ failure assessment (SOFA) scores and the number of systemic inflammatory response syndrome (SIRS) components fulfilled by 96 h following single time point paracetamol overdose. OLT, orthotopic liver transplantation.

Prospective validation of multiorgan failure scores

The prognostic accuracy of the SIRS and SOFA thresholds outlined above was then examined in a prospectively collected cohort of paracetamol-induced acute liver injury patients admitted to the Royal Infirmary of Edinburgh between March 2009 and July 2010. A total of 38 patients were included and their demographic details, admission laboratory parameters and clinical outcomes are shown in Table 5. All seven patients who died or underwent emergency OLT manifested a SIRS response, had a SOFA score >7 and had a modified SOFA >5 within 96 h of overdose. The specificity of the two SOFA predictors was 77.4 (95% CI 70.5–77.4) and the specificity of the SIRS was 74.2 (95% CI 67.2–74.2).

DISCUSSION

This study has explored the temporal relationship between paracetamol overdose and the development of systemic inflammation and organ failure. Development of the SIRS and extrahepatic organ failure are both strongly associated with adverse clinical outcomes and both SOFA and SIRS scores are highly sensitive markers of poor outcome following paracetamol overdose. A SOFA score ≤7 during the first 96 h post-overdose is highly predictive of spontaneous survival, with a risk of

Table 5 | Validation cohort of 38 single time point paracetamol overdoses

Variable (n = 38)		Value
Gender (male/female)		16/22 (42.1%/57.9%)
Age (years)		35 (26.5-44.5)
Received NAC in referring hospital		38 (100%)
Admission laboratory parameters	WCC ($\times 10^9$/L)	8.4 (6.5-11.4)
	Platelets ($\times 10^9$/L)	122 (71-192)
	Creatinine, μmol/L (mg/dL)	117 (61-241) (1.3 (0.7-2.7)
	ALT (IU/L)	5198 (2974-7978)
	Bilirubin, μmol/L (mg/dL)	81 (32-113) (4.7 (1.9-6.6)
	PT (seconds)	41 (29-73)
Ever encephalopathic (ALF)		18 (47.4%)
Grade 3-4 HE encephalopathy		12 (31.6%)
Mechanical ventilation		13 (34.2%)
KCC met		9 (23.7%)
SIRS response by 96 h post-overdose		15 (39.5%)
SOFA score >7 by 96 h post-overdose		14 (36.8%)
Modified SOFA score >5 by 96 h post-overdose		14 (36.8%)
Overall outcome		
Transplanted		4 (10.5%)
Spontaneous survival		31 (81.6%)
Died without transplantation		3 (7.9%)

Data are presented as median (± interquartile range) or percentages as appropriate.

death of <2%, and these patients also have a low risk of requiring specialist intracranial pressure monitoring or renal support. As a result of the high negative predictive values of both SOFA and SIRS, these scores may have a future role as 'gatekeepers' when considering patient transfer to tertiary liver centres.

Predicting individual prognosis accurately following paracetamol overdose is fraught with difficulty.[17] For a predictive model to be clinically useful, it should be easy to use, accurate, reproducible and accepted by clinical staff. Both SOFA and SIRS scores are dynamic tests, which can be rapidly recalculated throughout admission and are attractive as prognostic indicators because of their widespread use in intensive care units and their ease of use. Recently, the SOFA score was demonstrated to outperform liver-specific prognostic scores in predicting outcome among a cohort of critically ill chronic liver disease patients.[18] While both the SOFA and SIRS scores have similarly high negative predictive values following paracetamol overdose, the SOFA score is more attractive as a quantitative triage tool as, unlike the SIRS, it is an ordinal rather than dichotomous variable and therefore a rising SOFA score may help to identify deteriorating patients. Although neither SIRS nor SOFA were originally developed to predict outcome, the high sensitivity of these scores makes them attractive as a means of identifying all those high-risk paracetamol overdose patients who might die without transplant, although their relatively lower specificity suggests that they should not replace the KCC as definitive listing criteria. Additionally, there are some specific limitations of both SOFA and SIRS scores that should be addressed. The SIRS does not incorporate a hepatic failure component at all, while the hepatic failure component (serum bilirubin) of the SOFA score lacks specificity or the ability to differentiate acute from chronic hepatic dysfunction. Indeed, of all the six organ failure components combined in the SOFA score, only the hepatic failure element was not associated with an increased risk of death, a finding in keeping with several previous studies of this scoring system.[19, 20] Future studies should validate whether the modified SOFA score outlined above, which excludes hepatic organ failure, is a more accurate triage marker in this setting than the original SOFA score.

The principal strength of this study lies in its novel evaluation of SIRS and SOFA scores in relation to the timing of overdose, rather than in relation to the time of admission or the onset of HE. The initial symptoms of ALF are often vague and as a result there may be a significant delay between the overdose and subsequent hospitalisation. Additionally, some patients present as a result of the psychological consequences of their overdose rather than as a result of physical morbidity. Thirdly, as the majority of previous studies of prognostic variables of paracetamol-induced ALF have been performed at academic referral centres following patient transfer from smaller hospitals,[9] attempting to apply a prognostic score at the time of 'hospital admission' is considerably hindered by patient heterogeneity. Equally, accurately applying a prognostic score at the onset of HE may be problematic as the presence or absence of HE is subjective and subtle encephalopathy may be wrongly interpreted as resulting from alcohol or narcotic withdrawal. We have therefore attempted to minimise clinical heterogeneity by selecting single time point overdoses and have examined the evolution of systemic inflammation and organ failure in relation to this fixed time point. Using this approach, we demonstrate that the prognostic accuracy of both SIRS and SOFA scores are enhanced compared with previous reports which applied these markers at either the time of hospital admission or the onset of HE.[9] We recognise that this approach is dependent upon the temporal accuracy of the patients' overdose history, but previous studies have suggested that the patient history is usually reliable following paracetamol overdose.[21] Heterogeneity is further reduced by the single centre nature of this study, where both criteria for patient admission and clinical management protocols have remained unchanged during the time course of the study.

However, we recognise that this study has several important limitations. Clearly, the results of this study are not directly applicable to patients who have taken a staggered overdose, an increasingly recognised subtype of paracetamol overdose,[22] or in cases where accurate timings of the overdose are unavailable. Although we have examined the applicability of multiorgan failure scores in relation to time from overdose, patients access treatment at different time points in their clinical evolution, which may influence the development of the SIRS or organ failure. A further caveat is that some patients were transferred to the SLTU from a number of surrounding hospitals, each of which may have had different management protocols, clinical expertise and referral thresholds following paracetamol overdose. While the SLTU is the single referral point for all severe paracetamol overdoses in Scotland, we recognise the possibility of selection bias and that some patients with severe organ failure secondary to paracetamol overdose may not have been transferred to the SLTU.[23] We also recognise that not all of the patients in this study developed HE, and therefore ALF. However, predicting the development of HE can be equally problematic, and the mere absence of HE following severe acute liver injury does not preclude the development of other complications such as acute kidney injury.[24]

A potential application for SIRS and SOFA scores may be as gatekeepers to tertiary level care. In the SLTU, transfer criteria are based broadly upon guidelines previously published by the British Society of Gastroenterology.[12] These guidelines recognise that 'A significant proportion of cases will make an unremarkable recovery without intensive measures but this level of unnecessary transfer is justified if the potential for transplantation, in what is often a narrow window of time, is to be realised'. As a result of the rapid progression of paracetamol-induced ALF and the risks involved in transferring patients with HE or with incipient cerebral oedema, up to 50% of UK liver centre patients never develop HE. Developing sensitive markers to identify high-risk patients at an earlier time point, prior to HE developing, could accelerate safe patient transfer, but equally the high negative predictive value of the multiorgan scores outlined above could effectively rule out the need for emergency OLT by identifying those patients highly likely to survive with supportive care alone, thereby potentially avoiding patient transfer.

In conclusion, this retrospective cohort study of 100 severe acute paracetamol-induced acute liver injury patients has evaluated the incidence and temporal kinetics of both the SIRS and SOFA scores following single time point paracetamol overdose and demonstrates that these markers are highly sensitive at identifying patients with a poor prognosis. The high negative predictive value of these markers was validated in a prospective cohort. Both the SIRS and SOFA scores deserve further evaluation and external validation as potential gatekeepers to improve clinical decision-making models following paracetamol overdose.

ACKNOWLEDGEMENT

Declaration of personal and funding interests: None.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Admission laboratory parameters and clinical outcomes for study population. ALT, alanine aminotransferase; PT, prothrombin time.

Table S2. Risk factors for inhospital mortality at 72 and 96 h postparacetamol overdose: results of univariate analysis.

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